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Comparative analysis of inosine-substituted duplex DNA by circular dichroism and X-ray crystallography

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Abstract

Leveraging structural biology tools, we report the results of experiments seeking to determine if the different mechanical properties of DNA polymers with base analog substitutions can be attributed, at least in part, to induced changes from classical B-form DNA. The underlying hypothesis is that different inherent bending and twisting flexibilities may characterize non-canonical B-DNA, so that it is inappropriate to interpret mechanical changes caused by base analog substitution as resulting simply from ‘electrostatic’ or ‘base stacking’ influences without considering the larger context of altered helical geometry. Circular dichroism spectra of inosine-substituted oligonucleotides and longer base-substituted DNAs in solution indicated non-canonical helical conformations, with the degree of deviation from a standard B-form geometry depending on the number of I-C pairs. X-ray diffraction of a highly inosine-substituted DNA decamer crystal (eight I-C and two A-T pairs) revealed an A-tract like conformation with a uniformly narrow minor groove, reduced helical rise, and the majority of sugars adopting a C1'-*exo* (Southeastern) conformation. This contrasts with the standard B-DNA geometry with C2'-*endo* sugar puckers (South conformation). In contrast, the crystal structure of a decamer with only four I-C pairs has a geometry similar to that of the reference duplex with eight G-C and two A-T pairs. The unique crystal geometry of the inosine-rich duplex is noteworthy given its unusual CD signature in solution and the altered mechanical properties of some inosine-containing DNAs.

Keywords

A-tracts; B-DNA; circular dichroism; DNA topology; inosine; metal ion coordination; sequence-dependent helix geometry; X-ray crystallography

1. Introduction

The earliest X-ray diffraction studies of DNA fibers revealed two different flavors of DNA, an A form found at low water activity (~75% relative humidity) and a B form found at high

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Disclosure Statement

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water activity (Franklin & Gosling 1953; Watson & Crick 1953). Subsequent efforts have sought to understand the biological significance of duplex DNA polymorphism. On one level, DNA structural polymorphism manifests because of the ability of identical DNA sequences to favor different forms in different environments (solvents, ions, and ligands), with B-DNA the favored conformation under physiological conditions.

On a second level, different DNA sequences may adopt different preferred conformations under set conditions. The classic example is the left-handed Z-DNA conformation observed for some alternating purine-pyrimidine sequences (Wang et al. 1979). However, the interplay of base sequence, composition, structure, backbone flexibility, counterion condensation, and solvation in determining these conformations has been difficult to resolve. In one prominent model, DNA helical structure is determined by a ‘tug of war’ between the two grooves for cation localization (Hud & Polak 2001; Hud & Plavec 2003). Sequences with one groove that dominates the other for preferential cation localization tend to assume a helical structure other than the canonical B-form (e.g. B’-form or A-form), and sequence-directed axial curvature is connected to sequence-specific cation localization by a ‘cation-dependent junction model’ (Hud & Polak 2001; Hud & Plavec 2003). The interchange of sequence and shape is also key for biological function, e.g. the specific interaction of a protein with DNA requires recognition of the chemical signature of DNA sequence as well as intrinsic DNA structure (el Hassan & Calladine 1996; Okonogi, Alley, Reese, Hopkins & Robinson 2000; Harteis & Schneider 2014).

On yet a third level, recent work has revealed that B-DNA consists of two distinct nucleotide conformations, B-I and B-II (Imeddourene et al. 2016). The B-II subconformation comprises only about 10% of the B-DNA nucleotides in the Protein Data Bank (PDB) (Balaceanu et al. 2017). Yet, these structures differ from canonical B-I structures with respect to inclination and destacking of the bases, presence of a broad and very shallow major groove due to displacement of the bases towards the major groove, and presence of a deep and narrow minor groove (Hartmann, Piazzola & Lavery 1993; van Dam & Levitt 2000).

Biophysical and structural investigations of chemically modified DNA can provide insight into the underlying forces driving the properties of native DNA. In particular, nucleic acid base analogs have been utilized to investigate DNA mechanics and structure (Heath, Clendenning, Fujimoto & Schurr 1996; Berger, Tereshko, Ikeda, Marquez & Egli 1998; Ding, Gryaznov & Wilson 1998; Tereshko et al. 1998; Peters, Yelgaonkar, Srivatsan, Tor & James Maher 2013; Peters, Mogil, McCauley, Williams & Maher 2014). Dozens of high-resolution structures of chemically modified DNAs have been determined. One well-characterized nucleotide substitution is inosine for guanosine (i.e. the base hypoxanthine replacing guanine, removing the guanine exocyclic amino group). The resulting I-C base pairs have reduced stacking energy and form two hydrogen-bond interactions, rather than three in G-C base pairs. Hybridization studies suggest that the stabilities of inosine base pairs are $G \cdot C > I \cdot C > I \cdot A > I \cdot G \approx I \cdot T$ (Case-Green & Southern 1994; Janke, Riechert-Krause & Weisz 2011). Previous fiber diffraction studies have shown that poly[d(I)]·poly[d(C)] forms B-DNA, similar to the right-handed B-DNA form of poly[d(A)]·poly[d(T)] or poly[d(G)]·poly[d(C)] (Leslie, Arnott, Chandrasekaran & Ratliff 1980). These studies also examined poly[d(IC)], poly[d(AC)]·poly[d(IT)], poly[d(AI)]·poly[d(CT)], poly[d(AIT)]

·poly[d(ACT)], poly[d(AIC)]·poly[d(ICT)], and poly[d(IIT)]·poly[d(ACC)], revealing that, in many regards, I-C base pairs behave as A-T base pairs (Leslie et al. 1980). Under the appropriate conditions, these fibers can exist in several conformational families, i.e. A-, B-, and Z-DNA. This polymorphism has also been observed in solution using circular dichroism (CD) spectroscopy (Kyr, Kejnovska, Renciu & Vorlickova 2009). Poly[d(IC)] for instance, in addition to having A-DNA and B-DNA CD signatures under appropriate conditions (Vorlickova & Sagi 1991), has also been shown to display an unusual negative band at high wavelength indicating a left-handed helical structure (Mitsui et al. 1970) reminiscent of the CD spectrum of the left-handed Z-DNA of poly[d(G[5BrC])] (Fishel, Anziano & Rich 1990).

Our interest in the physical properties of I-substituted duplex DNA arose from our previous experiments to explore how replacement of thymine by charged and uncharged base analogs impacts the physical properties (melting temperature, helical geometry, bend and twist stiffnesses, etc.) of duplex DNA (Peters et al. 2013; Peters et al. 2014). This prior work was inspired by the proposal that DNA bend stiffness is dominated by DNA charge (Manning 2006), suggesting that charge-modified polymers should have distinct bending persistence lengths. In contrast, we were surprised to find that charged and uncharged base analogs had only small effects on bending persistence length, but larger effects on DNA twist flexibility measured in ligase-catalyzed cyclization experiments. Moreover, for an experimental ~400-bp sequence, substitution of some neutral base analogs (such as I for G) altered DNA CD spectra and increased double-helical DNA twist flexibility without large effects on global thermal stability or bend persistence length (Peters et al. 2013; Peters et al. 2014). These observations create a puzzle: what conformational differences might explain altered CD spectra and twist flexibility without large changes in thermal stability or bend persistence length for long I-substituted duplexes (Fig. 1)?

To further pursue this question, we here consider X-ray crystallography. The well-studied Dickerson-Drew dodecamer (DDD) is the classic example of B-DNA conformation (Drew et al. 1981; Tereshko, Minasov & Egli 1999; Dans et al. 2016). Based on the sequence of the self-complementary DDD, d(CGCGAATTCGCG), several I-containing dodecamers have previously been studied to determine the conformation of I-C and I-A base pairs in d(CG CIAATTCGCG) (Xuan & Weber 1992), d(CG CIAATTAGCG) (Corfield, Hunter, Brown, Robinson & Kennard 1987), and d(CG CAAATTIGCG) (Leonard, Booth, Hunter & Brown 1992), relative to the unmodified d(CGCGAATTAGCG) (Brown, Hunter, Kneale & Kennard 1986), d(CG CAAATTGGCG) (Brown, Leonard, Booth & Chambers 1989), and d(CG CAAATTTGCG) (Woods et al. 2004), respectively. The structures of the decamers d(CCAACGTTGG) and d(CCAAGATTGG) (Prive, Yanagi & Dickerson 1991) were also studied with I-C base pairs, i.e. d(CCAACITTGG) and d(CCAAIATTGG), respectively (Lipanov, Kopka, Kaczor-Grzeskowiak, Quintana & Dickerson 1993). Other published pairs of crystal structures (with and without I substitution) include the Holliday junction-forming d(CCIGTAC[5meC]GG) (Hays, Jones & Ho 2004) and the parent d(CCGGTAC[5meC]GG) (Vargason & Ho 2002); the A-DNA forming d(CCGGGCC[5meC]GG) (Tippin & Sundaralingam 1997) and its I variant d(CCIGGCC[5meC]GG) (Ramakrishnan & Sundaralingam 1995); the I-T wobble base pairs in the A-DNA octamer d(GGGGCTCC) (Hunter, Brown & Kennard 1986) and its I variant d(GGIGCTCC) (Cruse et al. 1989); and

d(CGCICICG) (Kumar, Harrison, Andrews & Weber 1992), which resembled the Z-DNA crystal structure of d(CGCGCG) (Gessner, Frederick, Quigley, Rich & Wang 1989; Malinina et al. 1998). Structures of I-containing duplexes that include other binding partners, e.g. proteins or the minor groove binder netropsin (Chen, Mitra, Rao, Sekar & Sundaralingam 1998; Shi, Mitra & Sundaralingam 2002), or without a reference structure were not considered here.

These cases create a database of examples showcasing the effects of I substitution on DNA crystal structures. However, the availability of high-resolution three-dimensional structures with corresponding solution CD spectra is limited. Expanding this collection is one objective of this work. The other motivation is to understand our previous observation that various base analog substitutions, including I, alter DNA conformation and physical properties as shown by CD spectroscopy and cyclization kinetics (Peters et al. 2013; Peters et al. 2014). We hypothesized that I-induced changes in DNA conformation would be detectable in X-ray crystallography experiments (Fig. 1).

Here we show that a high-resolution X-ray structure of a decamer DNA duplex with four I substitutions (four I-C pairs in place of G-C pairs) exhibits relatively close resemblance to the reference duplex with G-C and A-T pairs only, despite moderate differences between the solution CD spectra of the two duplexes. In contrast, the duplex with all eight G-C pairs replaced by I-C pairs adopts an A-tract like geometry in the crystal, with a minor groove that remains very narrow along the entire length of the decamer. Additional characteristics of this I-rich duplex include a 5% reduction in helical rise and Southeastern C1'-*exo* puckering by more than half the sugars. These features distinguish it from the reference duplex where we observe canonical B-form geometry, i.e. a 3.4 Å rise and mostly South C2'-*endo* puckered sugars. The unusual conformation of the I-rich duplex in the crystal may at least partially account for its non-B form character observed by CD spectroscopy in solution, even under crystallization conditions. We discuss the similarities between the I-rich duplex and A-tract DNA and the challenges of understanding properties of duplexes when comparing X-ray crystallography and CD spectroscopy in solution.

2. Materials and Methods

2.1 Oligonucleotides

All DNA oligonucleotides were purchased with standard desalting from either Integrated DNA Technologies (Coralville, IA) or Trilink Biotechnologies (San Diego, CA).

2.2 CD spectroscopy

CD spectroscopy was performed using a Jasco J-810 spectropolarimeter (Jasco). Briefly, ultraviolet-CD spectra were acquired from 320–210 nm, taking measurements every 0.5 nm with a scanning speed of 10 nm/min. Sample temperature was maintained at 4°C throughout, unless otherwise indicated. Samples were monitored three times with the average of the three scans reported. Samples were prepared by diluting ~7.5 nmol (~50 µg) of DNA into 300 µL of buffer and analyzed in a 0.1 cm cuvette. Buffer samples were scanned in a similar manner and these spectra subtracted from sample spectra.

Thermal denaturation experiments were conducted by sample heating from 5°C–85°C with a temperature ramp rate of 0.5°C/min. Wavelength scans between 320–210 nm were taken every 10°C at a data pitch of 1 nm, a scan rate of 100 nm/min, an accumulation number of 3, a bandwidth of 1 nm, and a response rate of 2 seconds. Single-wavelength data were collected every 0.5°C at the wavelength of a spectral maximum at 4°C.

All variable wavelength curves were smoothed using Friedman's super smoother (Friedman & Tibshirani 1984).

2.3 Thermal denaturation

Thermal denaturation data were processed following a two-state transition model (Mergny & Lacroix 2003). Briefly, the two states are referred to as “folded” (fully associated) and “unfolded” (fully dissociated) for simplicity. The data were converted from ellipticity (Θ) as a function of temperature (T) to fraction folded (θ) as a function of temperature

$$\theta(T) = \frac{\Theta(T) - u(T)}{f(T) - u(T)} \quad (1)$$

where the unfolded (u) and folded (f) baselines were determined from the data by linear regression.

First, a sigmoid model was fit to the fraction folded θ data

$$m(T) = \frac{1}{1 + e^{a(T-b)}} \quad (2)$$

In this model, $T_m = b$ since this is the value where $m(T) = 0.5$.

For the bimolecular reaction of self-complementary oligonucleotides ($2S \rightleftharpoons D$), the total strand concentration (C_t) is constant (and equal to the starting concentration of the DNA)

$$C_t = [S] + 2[D] \quad (3)$$

The fraction annealed or “folded” (i.e. duplex strand proportion of total strands) is

$$\theta = \frac{2[D]}{C_t} \quad (4)$$

so that the equilibrium constant is

$$K_a = \frac{[D]}{[S]^2} = \frac{\theta}{2C_t(1-\theta)^2} \quad (5)$$

and at the T_m it follows that $[S] = C_t/2$ and $[D] = C_t/4$, so that $K_a = 1/C_t$.

From the definition of Gibbs free energy

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (6)$$

and the well known relationship between Gibbs free energy and the equilibrium constant K_a

$$\Delta G^\circ = -RT\ln(K_a) \quad (7)$$

follows the classic form of the van't Hoff equation

$$\ln(K_a) = -\frac{\Delta H^\circ}{R}\left(\frac{1}{T}\right) + \frac{\Delta S^\circ}{R} \quad (8)$$

The change in enthalpy (ΔH°) and entropy (ΔS°) were determined from linear regression using this van't Hoff equation within the analysis interval ($0.2 < \theta < 0.8$) where the plots are reasonably linear. The T_m is calculated by rearranging this last equation when $K_a = 1/C_t$

$$T_m = \frac{\Delta H^\circ}{\Delta S^\circ + R\ln C_t} \quad (9)$$

2.4 Analysis of PDB files

Unless otherwise noted, conformational parameters of the DNA structures were determined from the PDB files using Web 3DNA (Lu & Olson 2003; Zheng, Lu & Olson 2009).

Molecular graphics images were rendered using open source PyMOL (Schrodinger 2015).

2.5 Crystallization, diffraction data collection and data processing

Crystals of I2G2 were grown by the hanging-drop vapor diffusion technique from droplets (2 μ L) containing oligonucleotide (0.23 mM), sodium cacodylate (25 mM, pH 6.5), magnesium acetate (12.5 mM), and 2-methyl-2,4-pentanediol (MPD, 20% v/v) that were equilibrated against a 1 mL reservoir of sodium cacodylate (50 mM, pH 6.5), magnesium acetate (25 mM), and MPD (40% v/v). I4 crystals were obtained from droplets (2 μ L) containing oligonucleotide (0.6 mM), sodium cacodylate (20 mM, pH 6.0), magnesium acetate (12.5 mM), and MPD (20% v/v) that were equilibrated against a 1 mL reservoir of sodium cacodylate (50 mM, pH 6.5), magnesium acetate (25 mM), and MPD (40% v/v). All crystals were mounted without further cryo-protection and flash-cooled in liquid nitrogen. I2G2 crystals were grown at 20°C and I4 crystals were grown at 4°C and mounted in the cold room as well.

Diffraction data were collected on the 21-ID-F or 21-ID-D beam lines of the Life Sciences Collaborative Access Team (LS-CAT) at the Advanced Photon Source (APS), located at

Argonne National Laboratory (Argonne, IL). For I2G2 crystals native data sets were collected on ID-F at 0.97625 Å. For I4 crystals, anomalous data sets were collected on 21-ID-D by tuning the wavelength to 0.9184 Å (bromine K edge). Crystals were kept at 100 K during data collection using MARCCD 225 or MARCCD 300 detectors. Diffraction data were integrated, scaled and merged with HKL2000 (Otwinowski & Minor). A summary of selected crystal data and data collection parameters is provided in Table 3.

2.6 Phasing and refinement

The I2G2 structure was solved by molecular replacement using the program MOLREP (Computational Project; Vagin & Teplyakov) and the native decamer d(CCAGGCCTGG) (G4, PDB ID 3U89) (Maehigashi et al.) as the starting model. The initial refinements were carried out using REFMAC5 (Collaborative Computational Project; Murshudov et al.) and water molecules were added after manually examining the electron density maps. The final refinements were carried out in SHELX (Schneider & Sheldrick). Ions were added after visualizing the electron density and their coordination geometries were examined carefully after each refinement cycle.

The I4 structure was phased by the single-wavelength anomalous dispersion (SAD) technique using the program PHENIX (Adams et al.). The resulting experimental electron density maps allowed visualization of 32 nucleotides of the 40 nucleotides per unit cell and AutoBuild (Terwilliger et al.) was used to build two partial duplexes. The initial orientations of the duplexes were optimized by rigid body refinement. Repeated rounds of difference Fourier synthesis in combination with manual building using the program COOT (Emsley & Cowtan) were performed to complete the duplex models. All refinements were carried out with the program REFMAC5 (Collaborative Computational Project; Murshudov et al.) and water molecules were added after inspecting sum and difference Fourier electron density maps. Water molecules and ions were gradually added as described for the I2G2 structure. Final refinement parameters are listed in Table 3.

2.7 Data deposition

Final coordinate and structure factor files for the I2G2 and I4 crystal structures have been deposited in the Protein Data Bank (www.rcsb.org): PDB ID codes 5W20 and 5W1Z, respectively.

3. Results and Discussion

3.1 CD signatures of long vs. short base-substituted duplexes: uracil and diaminopurine examples

Our study of inosine substitution was initially motivated by results of previous studies characterizing 417-base pair (bp) DNA duplexes with 54.2% GC content where one of the common natural bases was completely substituted by a DNA base analog (Peters et al. 2013; Peters et al. 2014). Modifications included seven charged and uncharged thymine analogs, or 2,6-diaminopurine (D) substituted for adenine, or hypoxanthine (the base of inosine, I) substituted for guanine (Peters et al. 2013; Peters et al. 2014). We did not observe meaningful differences in the absorbance spectra of these 417-bp substituted DNA

molecules (Fig. S1). Fig. 2A shows a comparison of CD spectra previously reported (Peters et al. 2013) for 417-bp natural (■), diaminopurine-substituted (Δ), and uracil-substituted (×) DNA. While the CD signature of uracil-substituted DNA is quite similar to that of natural DNA, the CD signature of diaminopurine-substituted DNA is distinct from both. The observed CD spectra could not be attributed to the mere presence of a modified residue dangling from the terminus of a model hexamer duplex (Fig. S2). We therefore interpreted these CD spectra as evidence of diaminopurine-dependent DNA polymorphism, indicating transitions to helical conformations different from canonical B-form DNA.

These results led to the hypothesis that even short (10–12 bp) DNA duplexes with base-substitutions might model the alternate DNA conformations implicated in the studies of 417-bp duplexes. To test this, we first examined the Dickerson-Drew dodecamer (DDD) composed only of the common natural bases. CD spectra for this parent molecule d(CGTGAATTCACG) (■) along with d(CGTGDDTTCDG) (Δ) and d(CGUGAAUUCACG) (×) are shown in Fig. 2B. The distinct CD signatures of these dodecamers are remarkably reminiscent of the longer substituted DNA molecules, indicating that the preferred helical conformations of each are conserved even in dodecamer duplexes.

3.2 CD signatures of 10-bp base-substituted duplexes: inosine examples

These previous studies focused on DNA molecules where the fraction of substituted bases ranged from 21% to 29%. We hypothesized that increasing the density of base modifications would more dramatically perturb the helical conformation of the resulting DNA. To address this question, we examined CD spectra of duplexes bearing an increasing number of G to I substitutions. The results are shown in Fig. 3. When compared to the unsubstituted parent decamer (bold, solid line), a single I substitution per strand (dotted line) does not substantially alter the observed CD spectrum. The spectral effect of two I substitutions per strand is dependent on whether the modifications are spaced (two dashed line) or adjacent (bold, dot dashed line). Finally, four I substitutions per strand (bold, long dashed line) resulted in the most altered CD spectrum. We ruled out differences in absorbance spectra (Fig. S3) or the contribution of CD spectra from unpolymerized nucleotides (Fig. S4) as possible explanations for these distinct CD spectra. This result is consistent with the hypothesis that increasing base substitution drives modified DNA molecules from the classical B-DNA conformation.

3.3 Inosine substitution effects on DNA stability

We sought to rule out that altered CD spectra might reflect duplex denaturation due to the destabilizing effects of I substitution. We therefore performed thermal denaturation experiments for I-substituted DNA molecules in 10 mM sodium cacodylate buffer (pH 6.6). Along with the parent, two decamer duplexes displaying the most unusual CD signatures at 4°C were chosen for further study: d(CCAGGCCTGG), d(CCAIICCTGG), and d(CCAIICCTII). CD spectra were collected at 10°C intervals from 5°C to 85°C (Fig. 4). Each of the decamers produced similar CD spectra in the denatured state at 85°C (Fig. 4D), further indicating that the duplex conformations are responsible for the observed CD spectra in the native state.

For each decamer, an appropriate peak wavelength was chosen for more detailed analysis (Table 1). CD ellipticity was collected as a function of temperature at this wavelength (Fig. 5A). Baselines were determined from the data by linear regression. Using equation 1 of Methods, the data were transformed to fraction folded (i.e. fraction annealed) as a function of temperature (Fig. 5B). The melting temperature (T_m), which occurs at a fraction folded of 0.5, was determined using two methods. First, a sigmoid model was fitted to the fraction folded data and the intersection point at 0.5 determined (Fig. 5B and Table 1). Second, the fraction folded data were further transformed to equilibrium constants (see equation 5 of Methods) and examined in the analysis interval (Fig. 5C). Fig. 5C shows a plot of the natural logarithm of the equilibrium constant as a function of inverse absolute temperature (in Kelvin) multiplied by one thousand. In the analysis interval, linear regression analysis and the van't Hoff equation were used to determine T_m (Table 1). The relative standard deviation of either method is about 2% (see Table S1). The agreement between the two methods in Table 1 is clearly within this range.

The melting temperature results are consistent with our previous studies showing that I-C pairs have a lower melting temperature than G-C pairs because I stacks onto adjacent base pairs with less energy than G (Peters et al. 2014). Here, we have shown that this effect is more striking in short decamer duplexes, with a melting temperature penalty of $\sim 8.8^\circ\text{C}$ per inosine substitution. However, even with this consideration, these data do not suggest that the altered CD spectra collected at the lowest temperatures are attributable to partial denaturation.

3.4 Crystallization buffer effects on CD spectra and CD-derived thermodynamic parameters

We set out to use X-ray crystallography to understand the origin of the perturbed CD spectra in modified duplexes. We first compared CD spectra obtained for the duplexes in standard buffer and in crystallization buffers. CD spectra for each duplex in the two buffers that yielded crystals are shown in Fig. 6. Unusual CD spectra tend to be preserved, though increasing concentrations of the precipitant ($\pm$)-2-methyl-2,4-pentanediol (MPD) reduce the magnitude of some CD spectral peaks. We conclude that crystallization buffers do not significantly alter the preferred conformations of the duplexes in solution.

We next performed thermal denaturation experiments for I-substituted DNA molecules in the two crystallization buffers to determine how these conditions affected thermal stability. Examples are shown in Fig. S5 (analysis in Fig. S6) and the results are summarized in Table 1. In all cases, the crystallization buffers stabilized the duplexes. Stabilization was most pronounced for d(CCAIICCTII) and inversely related to the initial stability of the duplex. These experiments showed that ionic conditions of the buffers stabilized the duplexes, even in the presence of the precipitant MPD.

3.5 Features of I-substituted DNA crystal structures in the PDB

We hypothesized that evidence of I-induced changes in DNA conformation would be detectable in pairs of modified and unmodified crystal structures previously reported in the PDB. Fig. 7 displays views of the central minor groove of structures of such B-DNA

duplexes superimposed with their I-substituted derivatives. Additional details of these structures appear in Table 2. The sets of structures are oriented to minimize root mean squared deviations (RMSD). Relative to the corresponding dodecamer references (with G instead of I), the RMSD of a duplex with central I·C pairs is 0.282 Å (453 atoms; Fig. 7A), with central I·A pairs RMSD = 0.377 Å (448 atoms; Fig. 7B), and with central A·I pairs RMSD = 0.444 Å (440 atoms; Fig. 7C). Rather than a reference with A·G pairs (Privé et al. 1987), using the B-DNA structure of the dodecamer with A·T pairs as the reference for this latest inosine variant gives RMSD = 0.611 Å (388 atoms; Fig. 7D). In a similar manner, relative to reference unmodified decamers, we have RMSD = 0.436 Å (370 atoms, Fig. 7E) with central I·C pairs and RMSD = 1.18 Å (402 atoms; Fig. 7F) with central I·A pairs. We conclude for these sets of structures with the parent DNA in B-form, that each variant structure with only one I substitution per strand closely resembles its unmodified partner.

In addition to the B-DNA sets, Fig. 8 shows superimposed structures of a Holliday junction, an A-DNA decamer, an A-DNA octamer with I·T wobble base pairs, and a left-handed duplex that resembles Z-DNA. This last example (Fig. 8D) is not a true set, because the I variant d(CGCICICG) is longer than the parent d(CGCGCG), but serves to show another type of helical conformation. RMSD = 0.898 Å (754 atoms, Fig. 8A) for the Holliday junction, RMSD = 0.307 Å (368 atoms, Fig. 8B) for the A-DNA decamer, and RMSD = 0.573 Å (320 atoms, Fig. 8C) for the A-DNA octamer. Again, each low density I-substituted structure largely resembles its partner.

Since major conformational differences were not obvious by inspection, we quantitatively analyzed main chain and glycosidic (χ) torsion angles, local base pair helical parameters, and local base pair step parameters using Web 3DNA (Vargason, Eichman & Ho 2000; Lu & Olson 2003; Anosova et al. 2016). Clusters of differing helical morphology emerge when using pairs of parameters, such as χ (the glycosidic torsion angle between the sugar and base) and δ (the backbone torsion angle associated with the sugar ring) (Anosova et al. 2016), as shown by the bounding ellipses in Fig. 10A. Each open circle in Fig. 10 corresponds to an individual base or base pair step from the B-DNA structures shown in Fig. 7 (black symbols) or the A-DNA structures in Fig. 8, B and C (red symbols), whereas the larger filled circles correspond specifically to the I-containing residues. Also shown are the previously reported bounding ellipses for the given parameters pairs (Lu & Olson 2003; Anosova et al. 2016). Fig. 10B shows z_p (the mean projection of the two phosphorus atoms onto the z-axis of the dimer step frame) and $z_p(h)$ (the mean projection of the two phosphorus atoms onto the z-axis of the helical reference frame). Fig. 10C shows x-displacement (the distance the midpoint of the short axis of a base pair is displaced from the helix axis) and inclination (the angle between the long axis of a base pair and a plane perpendicular to the helix axis). Fig. 10D shows slide (the in-plane displacement of one base pair relative to the previous along the short axis of the base pair step) and roll (the rotation of a base pair step about the long axis of the base pair step). In each case, there is good agreement with the previously reported discrimination between A-DNA and B-DNA (Lu & Olson 2003; Anosova et al. 2016). We conclude from this analysis of published structures that 8% to 10% I substitution does not drive altered DNA conformation as detected by crystallography. We do not know if the I-substituted DNA duplexes exhibited unusual CD spectra in solution in these cases.

3.6 CD spectra versus DNA crystal structures: new I-substituted examples

We therefore wished to analyze by X-ray crystallography our cases where I substitution clearly altered CD signatures. We first focused our attention on I2G2, the decamer d(CCAIICCTGG) whose CD signature appears as bold, dot dashed lines in Fig. 3. Crystals of I2G2 diffracted to ca. 1.3 Å and are of space group $P2_12_12_1$ with unit cell constants $34 \times 35 \times 41$ Å (Table 3). Fig. 10A shows an overlay of I2G2 superimposed with the G4 ‘native’ decamer (PDB code 3U89) (Maehigashi et al. 2012). The native G4 decamer is of space group $C2$. Therefore, incorporation of I appears to alter the packing arrangement, but not the overall structure of the duplex. The minor groove of the I2G2 decamer is only slightly narrower (Fig. 10C). One complication arises from conformational disorder in the G4 decamer, where for many nucleotides two alternative conformations were refined (Maehigashi et al. 2012). We deleted atoms of the “B” structure and only retained the “A” structure for the overlay. The RMSD for 316 atom pairs is 0.878 Å. This novel structure demonstrates that for a DNA duplex with 20% base substitution and clearly distinct helical conformation from native DNA by CD in solution (Fig. 3), the resulting crystal structure largely does not reveal the alternate DNA conformation.

To extend this work, we studied I4, the decamer with the highest degree of base substitution (40%) and the most distinctive helical geometry by solution CD (bold, long dashed lines in Fig. 3). We obtained the crystal structure of the I4 decamer phased by bromine single-wavelength anomalous dispersion (Br-SAD) (Egli et al. 1998; Egli, Lubini & Pallan 2007; Egli 2010). This crystal form is of space group $P1$, contains two duplexes per asymmetric unit, and the resolution is 1.53 Å (Table 3). Unlike in the case of the G4 and I2G2 duplexes, the overlay of the G4 and I4 duplexes (Fig. 10B) reveals a considerably different geometry for the I4 helix. The most obvious distinction relates to minor groove width, which remains very narrow along the entire length of the I4 decamer (Fig. 10C).

Symmetric DNA A-tracts are generally defined as DNA sequences of at least four consecutive A·T base pairs without an intervening T·A step, whereas asymmetric A-tracts are generally A_nT_m , although two or more As followed by pyrimidines, A_nY_m , are referred to as an A-tract in addition to homo-A-homo-T runs. When embedded in a general sequence, A-tracts centrally curve the DNA double helix towards the minor groove, which progressively narrows in the 5′ to 3′ direction of the adenine strand (Drsata et al. 2014). A major finding of previous A-tract crystal structures is that the minor groove is exceptionally narrow (Shatzky-Schwartz et al. 1997). As the minor groove of I4 is contracted, it could be referred to as a DNA ‘AI’-tract in analogy to A-tract DNA. In fact the minor groove remains narrow up to the very 3′ terminus (II) of the strand (Fig. 10B). This cooperative behavior by sequential I residues may also partly explain the two different CD spectra observed for two I substitutions per strand in Fig. 3, whereby the CD spectra were dependent on whether the two modifications were spaced (two dashed line) or adjacent (bold, dot dashed line) to one another.

Besides a narrow minor groove, the hallmark of an A-tract in solution, and especially phased A-tracts, is the induced curvature of the helix axis. Gel electrophoresis experiments demonstrate that apparent curvature is largely retained when I substitutions are introduced singly into A-tracts but decreases abruptly for pure I-tracts. One I substitution does not cause

any detectable change in curvature at the 5' terminus of an A-tract (e.g. comparing AAATTT with IAATTC), it reduces curvature by 10% to 12% within an A-tract (e.g. AAIAA or AIATCT) (Diekmann, von Kitzing, McLaughlin, Ott & Eckstein 1987; Koo & Crothers 1987; Shatzky-Schwartz et al. 1997), and exhibits the greatest effect at the 3' terminus of the A-tract (Mollegaard, Bailly, Waring & Nielsen 1997). Global axis curvature strongly differs between G4 (0.5°) and I4 (10.4°). This and other differences in helical parameters that distinguish the G4 and I4 duplexes were calculated with the program CURVES [63]. For instance, in the G4 duplex the average helical rise (global inter-base pair parameter) is 3.44 Å (max. 3.75 Å, min. 3.14 Å), but it is reduced in the I4 duplex to 3.29 Å (max. 3.55 Å, min. 3.07 Å).

Systematic differences in the sugar puckers are also noteworthy. More than half of the 2'-deoxyribose sugars (11 of 20) in the I4 duplex display a Southeastern pucker mode (C1'-exo). By comparison, only five sugars in the G4 reference duplex exhibit such a pucker. It is interesting that half of the eight A-tract residues (underlined) in the central portion of the DDD duplex d(CGCGAAATTCGCG) also adopt the C1'-exo pucker (PDB ID 355D) (Shui, McFail-Isom, Hu & Williams 1998). However, unlike in the DDD and G4 duplexes, the minor groove in I4 remains narrow throughout and becomes even narrower at the duplex ends (Fig. 10C). With respect to d(CCAIICC[5BrU]II), the I4 decamer features a central AI-tract (underlined) that apparently zips up the minor groove, and this extremely narrow minor groove is among the hallmarks of an I-rich duplex.

Interestingly, waters form a partial spine in the center of the minor groove of I4. The metal ion coordination of both I2G2 and I4 can be seen together with the quality of the electron density in Fig. 11. Fig. 11A shows the density for I2G2 around Mg²⁺ ions in the major and minor groove on opposite sides of the same I-C pair, and Fig. 11B shows the density for I4 around the Na⁺ coordinated to O2 oxygens of C residues on opposite strands at the central base pair step. Fig. 11C depicts the metal ions bound in the major and minor grooves of the three duplexes. The view is into the major groove, G4 is at the left, I2G2 is in the middle and I4 is at the right. Filled circles in red indicate Mg²⁺ hexahydrate ions in the major groove and open circles indicate Mg²⁺ in the minor groove. G4 occupies a crystallographic dyad and the three open circles next to T and C in the minor groove indicate three partially occupied ions (A, B and C in PDB 3U89). The coordinations of Mg²⁺ ions change in I2G2 so that they are grouped around central I-C pairs in both grooves, indicating that the minor groove is still wide enough to accommodate a Mg²⁺ hexahydrate in that duplex (the asymmetric unit in the I2G2 structure contains one duplex in a general location). Finally, the I4 duplex shows Mg²⁺ hexahydrates as well as Na⁺ (open cyan circles indicate minor groove coordination). However, the central minor groove in I4 is too narrow to accommodate a Mg²⁺ ion. Instead, Mg²⁺ ions are positioned at the ends of the duplex that leave more space for the hexahydrate ion. Na⁺ is coordinated at the center of the minor groove and bridges O2 oxygens of Cs from opposite strands. Alkali metal ions make comparable contacts at central T residues in the minor groove of A-tracts, e.g. AATT (Tereshko, Minasov & Egli 1999). This observation further underscores the similarity between A- and AI-tracts. Na⁺ are smaller and they are not fully hydrated compared to Mg²⁺, i.e. they form inner-sphere complexes. In addition a Na⁺ ion lies adjacent to BrU in the minor groove. Because I4

features two independent duplexes, Fig. 11C depicts ion position information from the two minor grooves in one duplex.

The unusual conformation of I4 is intriguing considering the unique pattern of metal ion coordination. Metal ion interactions may play a key role in stabilizing this DNA conformation. It is possible that disruption of groove hydration (e.g. by dehydrating agents) could drive a transition to a more conventional DNA conformation, reducing AI-tract-dependent curvature.

4. Summary and Conclusions

Motivated by our previous finding based on CD spectroscopy that base analogs can drive new DNA conformations with non-canonical physical properties, we wished to characterize these non-B conformations (Fig 1). Specifically, we hypothesized that X-ray crystallography would reveal inosine-driven changes in DNA duplex helical conformation. A literature review showed that single-I or double-I substitutions typically preserve conventional A-DNA and B-DNA in crystal structures. From CD experiments it is apparent that complete G to I substitution induced unusual DNA conformations in longer constructs, and this was observed as well in decameric DNA duplexes, with CD changes becoming more pronounced for duplexes with higher numbers of I substitutions (e.g. I4 duplex vs. I2G2 duplex). Our crystallography experiments demonstrate that the I2G2 decamer with two I substitutions per strand and four I-C pairs per duplex adopts a conformation that is similar to that of the G4 reference duplex without inosine. However, the crystal structure of the I4 decamer with four I substitutions per strand and eight I-C pairs per duplex displays striking changes relative to the conformation of the G4 duplex. Distinguishing features include base pair rise, sugar conformation, axis curvature, groove topology, and groove occupancy by water and ions. To the extent that the duplex with almost all base pairs replaced by I-C shows a marked change in conformation relative to canonical B-form DNA (e.g. G4 duplex), the crystallographic data are consistent with results from solution studies wherein increasing levels of substitution trigger more pronounced deviations in the CD spectra. However, the X-ray structure still lies within the B-DNA family of conformations (c.f. the blue symbols in Fig. 10). Based on the I4 crystal structure, we can conclude that hallmarks of an I-rich duplex include an extremely narrow minor groove, a contraction in length as a consequence of overall more negative inclination, and a modest curvature of the calculated helical axis in the decamer. How these properties might contribute to the observed changes in the CD spectra of I-substituted DNA duplexes in solution remains an open question.

This project was motivated by an analysis of DNA mechanics where we assume that DNA conformation is homogenous and without anomalous junctions between DNA segments of altered conformation (Schurr, Delrow, Fujimoto & Benight 1997). It is formally possible that unusual CD spectra for base-substituted DNAs are due to isolated segments of altered conformation, with intervening junctions that might explain the observed changes in mechanical properties. However, we reasoned that any such junctions would manifest through substantial reduction of melting temperature and sites of enhanced bend flexibility through kinking. This was not observed (Peters et al. 2013; Peters et al. 2014), nor was there

evidence of spontaneous kinking in base-substituted DNA molecules ~750 bp long visualized using atomic force microscopy (Peters et al. 2014).

The necessarily static picture of DNA derived from X-ray crystallography studies is contrary to the many experimental and theoretical studies that reveal that DNA is highly polymorphic and dynamic. Indeed, divalent metal ions and packing forces play a significant role in the structure of short DNA duplexes determined by crystallography (Minasov, Tereshko & Egli 1999). For instance, when the structure of the A-DNA decamer d(GCGGGCCCCG) was studied in two different crystal forms (Ramakrishnan & Sundaralingam 1993), the authors concluded that the crystal environment dominated base sequence effects on DNA conformation (Ramakrishnan & Sundaralingam 1993; Malinina et al. 1998). However, study of the same DNA sequence in different crystal packing arrangements can provide useful insight into structural variability. This conundrum is analogous to the challenges of X-ray crystallography applied to understand apparent DNA curvature induced by A-tract sequences. These crystal structures showed more straight conformations, whereas solution nuclear magnetic resonance (NMR) structures of A-tract DNA showed more pronounced helix curvature, consistent with the extensive evidence from anomalous gel mobility experiments of phased A-tracts (Brahms & Brahms 1990; Young et al. 1995; MacDonald, Herbert, Zhang, Polgruto & Lu 2001; Barbi, Zimmer & Crothers 2003; Stefl, Wu, Ravindranathan, Sklenár & Feigon 2004). Despite this limitation that crystal-packing or lattice effects tend to suppress differences in helical geometry detected by NMR or CD in solution, X-ray analysis has demonstrated that alternating purine regions, e.g. AIA, are structurally similar to pure A-tracts (Shatzky-Schwartz et al. 1997), consistent with our findings for the I-rich duplex. Moreover, differences in the behavior of I-C versus A-T base pairs became more pronounced as additional I substitutions were made, consistent with our CD and crystal structure data. Additionally, X-ray analysis of the I-tract-containing decamer d(CCIHCCCGG), showed that runs of inosines share the structural feature of a narrow minor groove with A-tracts (Shatzky-Schwartz et al. 1997).

Here we have shown that high-resolution X-ray structures of short DNA duplexes with moderate I substitution preserve B-form DNA features in crystals, despite the fact that in solution these molecules exhibit non-B form character by CD spectroscopy, even under crystalizing conditions. Moreover, highly I-substituted oligonucleotides with pronounced non-canonical helical conformations apparent by CD displayed altered helical conformations that were less pronounced by crystallography. High-resolution structural methods other than X-ray crystallography may be necessary to fully resolve this ongoing mystery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Overall strategy. A. Previous work characterized the mechanical properties of DNA molecules greater than 400 bp in length and incorporating DNA base analogs such as inosine (Peters et al. 2013; Peters et al. 2014). These DNA molecules exhibited altered DNA twist flexibilities that correlated with altered circular dichroism (CD) spectra (Peters et al. 2013). We hypothesized that altered CD spectra reflected altered helical conformation and sought to test if altered helical conformations were detected by X-ray crystallography. A. DNA containing only the four common natural bases (top) displays a conventional B-DNA helical signature in CD experiments (middle). Structural studies of short DNA duplexes (10–12 bp) allow direct visualization of this canonical B-form DNA conformation (bottom). B. DNA molecules incorporating modified bases (red R) display altered twist flexibility (top), correlating with altered CD spectra (middle). This work explores whether the implied altered helical conformations are detectable in the crystal structures of inosine-substituted DNA (bottom).

Figure 2.

CD spectroscopy showing ellipticity (Θ) as a function of wavelength (λ). A. CD spectra previously reported (Peters et al. 2013) for 417-bp DNA molecules collected in 10 mM phosphate buffer, pH 7.0, containing 1 M NaCl and comparing natural (■), diaminopurine-substituted (△), and uracil-substituted (×) DNA. B. CD spectra of dodecamers collected in 10 mM sodium cacodylate, pH 6.6, and comparing natural (■), diaminopurine-substituted (△), and uracil-substituted (×) DNA.

Figure 3.

CD spectroscopy of inosine-substituted DNA showing ellipticity (Θ) as a function of wavelength (λ). CD spectra of a series of decamers with increasing number of I substitutions collected in 10 mM sodium cacodylate, pH 6.6. When compared to the unsubstituted parent decamer (bold, solid line), a single I substitution (dotted line) does not substantially alter the observed CD spectrum. The effect of two I substitutions on the observed CD spectra is dependent on whether the modifications are spaced (two dashed line) or adjacent (bold, dot dashed line). Four I substitutions of the parent decamer (bold, long dashed line) result in the most altered CD spectrum.

Figure 4.

CD thermal denaturation analysis of inosine-substituted DNA molecules in 10 mM sodium cacodylate, pH 6.6. CD spectra showing ellipticity (Θ) as a function of wavelength (λ) were collected at 10°C intervals from 5°C to 85°C (distinguished by color) for A. d(CCAGGCCTGG) duplex (solid lines), B. d(CCAIICCTGG) duplex (dot dashed lines), and C. d(CCAIICCTII) duplex (long dashed lines). D. Comparison of the decamers in the denatured state (85°C), distinguished as above.

Figure 5.

Processing of CD thermal denaturation data for inosine-substituted DNA molecules. A. Representative single wavelength CD ellipticity collected as a function of temperature for the d(CCAGGCCTGG) duplex followed at 235 nm. Baselines (solid lines) were determined from the data by linear regression. B. Using equation 1 of Methods, data were transformed to fraction folded (i.e. fraction annealed) as a function of temperature. The melting temperature (T_m) occurs at a fraction folded of 0.5 (horizontal dashed line). Two methods were used to determine the T_m . First, a sigmoid model (bold curve) was fitted to the fraction folded data and the intersection point at 0.5 determined. Second, the fraction folded data were further transformed to equilibrium constants (equation 5 of Methods) and examined in the analysis interval (vertical lines). C. The natural logarithm of the equilibrium constant as a function of inverse absolute temperature (in Kelvin) multiplied by one thousand. In the analysis interval (vertical lines), linear regression analysis (bold line) and the van't Hoff equation were used to determine T_m (see Methods).

Figure 6.

CD spectroscopy of inosine-substituted DNA molecules in various buffers. CD spectra showing ellipticity (Θ) as a function of wavelength (λ) were collected in 10 mM sodium cacodylate, pH 6.6 (black), 50 mM sodium cacodylate, pH 6.5; 25 mM magnesium acetate; 40% MPD (blue), or 40 mM sodium cacodylate, pH 5.5; 20 mM magnesium chloride; 10% MPD; 40 mM lithium chloride; 20 mM cobalt hexamine chloride (red) for A. d(CCAGGCCTGG) duplex (G4, solid lines), B. d(CCAIICCTGG) duplex (I2G2, dot dashed lines), and C. d(CCAIICCTII) duplex (I4, long dashed lines). Due to high HT voltages for wavelengths less than 250 nm in crystallization buffer 2, no data were collected below 240 nm.

Figure 7.

Crystal packing of B-DNA duplexes with their inosine-substituted derivatives (see Table 2). I nucleobases are highlighted in magenta. A. Superimposition of d(CGCGAATTCGCG) (blue; PDB code 7BNA) (Wing et al. 1980; Holbrook, Dickerson & Kim 1985) and d(CGCAATTCGCG) (green; PDB code 1D77) (Xuan & Weber 1992) duplexes, corresponding to the relative orientation with the smallest root mean squared deviation. B. Superimposition of d(CGCGAATTAGCG) (forest; PDB code 112D) (Brown et al. 1986) and d(CGCAATTAGCG) (red; PDB code 114D) (Corfield et al. 1987). C. Superimposition of d(CGCAAATTGGCG) (dark blue; PDB code 111D) (Brown et al. 1989) and d(CGCAAATTIGCG) (orange; PDB code 1D81) (Leonard et al. 1992). D. Superimposition of d(CGCAAATTTGCG) (cyan; PDB code 111D) (Woods et al. 2004) and d(CGCAAATTIGCG) (orange; PDB code 1D81) (Leonard et al. 1992). E. Superimposition

of d(CCAACGTTGG) (gray; PDB code 5DNB) (Prive et al. 1991) and d(CCAACITTGG) (salmon; PDB code 1D61) (Lipanov et al. 1993). F. Superimposition of d(CCAAGATTGG) (lime green; PDB code 3DNB) (Prive et al. 1991) and d(CCAAIATTGG) (light pink; PDB code 1D62) (Lipanov et al. 1993).

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Figure 8.

Crystal packing of non-B form duplexes with their inosine-substituted derivatives (see Table 2). I nucleobases are highlighted in magenta. A. Superimposition of 4-way junction formed from d(CCGGTAC[5meC]GG) (light blue; PDB code 1L6B) (Vargason & Ho 2002) and d(CCIGTAC[5meC]GG) (yellow; PDB code 1S1L) (Hays et al. 2004). B. Superimposition of the A-DNA decamers d(CCGGGCC[5meC]GG) (violet, PDB code 323D) (Tippin & Sundaralingam 1997) and d(CCIGGCC[5meC]GG) (limegreen, PDB code 213D) (Ramakrishnan & Sundaralingam 1995). C. Superimposition of the A-DNA octamers

d(GGGGCTCC) (brown; PDB code 1D92) (Hunter et al. 1986) and d(GGIGCTCC) (marine blue; PDB code 1D90) (Cruse et al. 1989). D. Superimposition of the Z-DNA d(CGCGCG) (white; PDB code 392D) (Malinina et al. 1998) and d(CGCICICG) (bright orange; PDB code 1D53) (Kumar et al. 1992).

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Figure 9.

Plots of pairs of selected conformational parameters showing the differences between A-DNA (red) and B-DNA (black). For comparison, the parameters for the new I4 structure are shown in blue. Parameters for natural base pairs or base pair steps are shown with open circles, whereas the larger filled circles correspond specifically to the I-containing residues. The bounding ellipses were previously reported (Lu & Olson 2003; Anosova et al. 2016). A. Torsion angles δ and χ . B. Projected phosphorous positions, z_P and $z_P(h)$. C. Helical inclination and x -displacement. D. Dimer step roll and slide.

Figure 10.

Crystal structures of current inosine-substituted derivatives (see Table 2). I nucleobases are highlighted in magenta. A. Superimposition of d(CCAGGCCTGG) (G4, beige; PDB ID 3U89) (Maehigashi et al. 2012) and d(CCAIICCTGG) (I2G2, purple) duplexes, corresponding to the relative orientation with the smallest root mean squared deviation. B. Superimposition of d(CCAGGCCTGG) (G4, beige) and d(CCAIICC[5BrU]II) (I4, deep teal) demonstrates the decreased overall length of the latter duplex. C. Minor groove width (solid lines) and depth (dashed lines) in Å for the G4 (beige), I2G2 (purple) and I4 (deep teal) duplexes, illustrating the significantly contracted minor groove in the I4 duplex. The two parameters were calculated with the program CURVES (Lavery & Sklenar 1996).

Figure 11.

Quality of the electron density in the crystal structures of the I2G2 and I4 duplexes and comparison of the metal ion coordination in the grooves of the G4, I2G2 and I4 duplexes. Final Fourier 2Fo-Fc sum electron density (1.2σ level) in the A. I2G2 and B. I4 crystal structures. The I2G2 duplex is viewed across the major (left) and minor (right) grooves, with Mg^{2+} (red spheres of larger radius) hexahydrate complexes coordinating on both sides of an I-C pair in the central portion. The I4 duplex is viewed into the minor groove with a Na^+ ion (cyan sphere) invading the water spine and coordinating to O2 atoms of cytosine residues

from opposite strands at the central d(IpC)-d(IpC) base pair step. C. Schematic illustration of metal ion coordination inside the grooves of the native G4 duplex and the I-substituted I2G2 and I4 duplexes (from left to right). The view is into the major groove, filled circles in red indicate Mg^{2+} hexahydrate ions inside the major groove, and open red and cyan circles indicate Mg^{2+} and Na^+ , respectively, inside the minor groove. G4 sits on a crystallographic dyad and the three open circles next to T and C in the minor groove indicate three partially occupied ions (labeled A, B and C in PDB 3U89). The asymmetric unit of the I2G2 structure contains one duplex in a general location. The asymmetric unit of the I4 structure contains two independent duplexes and the schematic at the right depicts a composite of the ion coordination to these two duplexes, i.e. ions coordinating to them were combined inside the minor groove of a single duplex.

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Table 1.CD-derived thermal stabilities.^a

Sequence	λ (nm)	Buffer	T_m (°C) ^b	T_m (°C) ^c
CCAGGCCTGG	235	10 mM sodium cacodylate, pH 6.6	51.9	52.1
		Crystallization buffer #1 ^d	52.8	52.9
		Crystallization buffer #2 ^e	55.2	55.9
CCAHCCTGG	280	10 mM sodium cacodylate, pH 6.6	34.2	33.6
		Crystallization buffer #1 ^d	37.0	37.0
		Crystallization buffer #2 ^e	39.8	39.9
CCAHCCTII	265	10 mM sodium cacodylate, pH 6.6	9.6	9.6
		Crystallization buffer #1 ^d	16.5	16.3
		Crystallization buffer #2 ^e	21.9	22.5

^aRelative standard deviation from 3–4 repeats is 2% for T_m estimates (see Table S1).^bUsing a sigmoid fit method (see Methods for details).^cUsing the van't Hoff method (see Methods for details).^d50 mM sodium cacodylate, pH 6.5; 25 mM magnesium acetate; 40% MPD.^e40 mM sodium cacodylate, pH 5.5; 20 mM magnesium chloride; 10% MPD; 40 mM lithium chloride; 20 mM cobalt hexamine chloride.

Table 2.

Selected structural parameters for DNA duplexes.

Duplex	PDB ID	Length (bp)	Space Group	Resolution (Å)	Type	Ref.
CGCGAATTCGCG	7BNA	12	$P2_12_12_1$	1.9	B	(Wing et al. 1980; Holbrook et al. 1985)
CGCIAATTCGCG	1D77	12	$P2_12_12_1$	2.4	B	(Xuan & Weber 1992)
CGCGAATTAGCG	112D	12	$P2_12_12_1$	2.5	B	(Brown et al. 1986)
CGCIAATTAGCG	114D	12	$P2_12_12_1$	2.5	B	(Corfield et al. 1987)
CGCAAATTGGCG	111D	12	$P2_12_12_1$	2.25	B	(Brown et al. 1989)
CGCAAATTIGCG	1D81	12	$P2_12_12_1$	2.5	B	(Leonard et al. 1992)
CGCAAATTTGCG	1S2R	12	$P2_12_12_1$	1.53	B	(Woods et al. 2004)
CCAACGTTGG	5DNB	10	$C2$	1.4	B	(Prive et al. 1991)
CCAACITTGG	1D61	10	$C2$	1.3	B	(Lipanov et al. 1993)
CCAAGATTGG	3DNB	10	$C2$	1.3	B	(Prive et al. 1991)
CCAAIATTGG	1D62	10	$C2$	2.0	B	(Heinemann, Alings & Hahn 1994; ElHassan & Calladine 1997)
CCGGTAC[5meC]GG	1L6B	10	$C2$	1.5	Holliday	(Vargason & Ho 2002)
CCIGTAC[5meC]GG	1S1L	10	$C2$	2.2	Holliday	(Hays et al. 2004)
CCGGGCC[5meC]GG	323D	10	$P2_12_12_1$	2.15	A	(Tippin & Sundaralingam 1997)
CCIGGCC[5meC]GG	213D	10	$P2_12_12_1$	1.6	A	(Ramakrishnan & Sundaralingam 1995)
GGGGCTCC	1D92	8	$P6_1$	2.25	A	(Hunter et al. 1986)
GGIGCTCC	1D90	8	$P6_1$	1.7	A	(Cruse et al. 1989)
CGCGCG	392D	6	$C222_1$	3.0	Z	(Malinina et al. 1998)
CGCICIG	1D53	8	$P6_5$	1.5	Z	(Kumar et al. 1992)
CCAGGCCTGG	3U89	10	$C2$	0.96	B	(Maehigashi et al. 2012)
CCAIICCTGG	5W20	10	$P2_12_12_1$		B	this work
CCAIICC[5BrU]II	5W1Z	10	$P1$		B	this work

Table 3.

Selected crystal data, diffraction data collection and refinement parameters.

Crystal structure	I2G2	I4
Unit cell constants <i>a</i> , <i>b</i> , <i>c</i> [Å]; α , β , γ [°]	34.19, 35.04, 41.34 90.0, 90.0, 90.0	24.88, 32.80, 34.03 90.25, 107.32, 111.04
Space group	<i>P</i> ₂ ₁ ₂ ₁	<i>P</i> ₁
Data collection		
Wavelength [Å]	0.97625	0.9184
Resolution (outer shell) [Å]	50.00 – 1.36 (1.41 – 1.36)	32.24 – 1.55 (1.58 – 1.55)
Unique reflections	11,019 (1,002)	13,618 (640)
Completeness [%]	99.1 (95.0)	97.1 (90.0)
<i>I</i> / σ (<i>I</i>)	30.57 (5.20)	28.70 (2.37)
R-merge	0.073 (0.343)	0.073 (1.000)
R-pim	0.035 (0.133)	0.053 (0.434)
Redundancy	7.7 (7.6)	5.9 (4.5)
Refinement		
R-work	0.184	0.207 (0.316) ^a
R-free	0.250	0.234 (0.364) ^a
Number of nucleic acid atoms ^b	437	803
Avg. B-factor: nucleic acid atoms [Å ²]	10.6	22.7
Avg. B-factor: Mg ²⁺ / Na ⁺ / water [Å ²]	14.8 / -- / 26.1	28.1 / 32.1 / 28.7
R.m.s.d. bond lengths [Å]	0.0148	0.012
R.m.s.d. bond angles ^c	0.03 [Å]	2.1 [°]
PDB ID	5W20	5W1Z

^aRefinement outer shell 1.57–1.55 [Å]^bIncludes dual occupancy atoms^cUnits are different because of the different software used for refinement: in REFMAC5 r.m.s.d. angles are in degrees and in SHELX they are in Å (1...3 distances)