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Crystal structure of a B-DNA dodecamer containing inosine, d(CGCIAATTCGCG), at 2.4 Å resolution and its comparison with other B-DNA dodecamers

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ABSTRACT

The crystal structure of the dodecamer, d(CGCIAATTCGCG), has been determined at 2.4 Å resolution by molecular replacement, and refined to an R-factor of 0.174. The structure is isomorphous with that of the B-DNA dodecamer, d(CGCGAATTCGCG), in space group $P2_12_12_1$ with cell dimensions of a = 24.9, b = 40.4, and c = 66.4 Å. The initial difference Fourier maps clearly indicated the presence of inosine instead of guanine. The structure was refined with 44 water molecules, and compared to the parent dodecamer. Overall the two structures are very similar, and the I:C forms Watson – Crick base pairs with similar hydrogen bond geometry to the G:C base pairs. The propeller twist angle is low for I4:C21 and relatively high for the I16:C9 base pair (–3.2° compared to –23.0°), and the buckle angles alter, probably due to differences in the contacts with symmetry related molecules in the crystal lattice. The central base pairs of d(CGCIAATTCGCG) show the large propeller twist angles, and the narrow minor groove that characterize A-tract DNA, although I:C base pairs cannot form the major groove bifurcated hydrogen bonds that are possible for A:T base pairs.

INTRODUCTION

Inosine is a purine nucleoside whose neutral base, hypoxanthine, forms stable base pairs with all four conventional bases, and the strength of the base pairing is approximately equal in each case (1). Inosine (I) occurs naturally in the wobble position of the anticodon of some t-RNA's, where it appears to pair with adenosine in addition to cytidine and uridine, the nucleosides that pair with guanosine in that position. Poly(rI) and poly(dI) form stable helices with poly(rC) and poly(dC) (2), and serve as templates for the incorporation of cytosine into products of DNA and RNA polymerases (3). Oligonucleotides containing inosines have been used extensively as hybridization probes to screen human cDNA or genomic DNA libraries when cloning genes for proteins containing amino acids with degenerate codons (4).

Despite the widespread use of inosine, there have been relatively few studies of the effect of inosine on the atomic structure of oligonucleotides. Moreover, polymers of DNA containing inosine occur in various conformations, including right-handed and left-handed double helices and quadruple helices as measured by fiber diffraction and circular dichroism experiments (5; 6; 7). Circular dichroism spectra and X-ray fiber diffraction have indicated that poly d(I-C).poly d(I-C) formed a left-handed helical structure (8; 9). In contrast, fiber diffraction experiments have shown that poly dI.poly dC formed B-DNA double helices (10). More recently, Vorlickova and Sagi (7) have studied the effects of salt concentration on the conformation of poly d(I-C) using circular dichroism spectroscopy, and have observed A-, B-, and Z-DNA conformations, as well as an unusual conformation at low salt.

The hypoxanthine base of inosine resembles guanine without the 2-amino group. Base pairing between I and C is possible by forming two hydrogen bond interactions as in A:T base pairs, instead of the three that occur in C:G base pairs. The I:C base pairs are expected to resemble A:T in the minor groove, and G:C in the major groove of B-DNA in the arrangement of potential hydrogen bond donors and acceptors available for interaction with proteins. Substitution of I:C for A:T base pairs has been used to test whether transcription factor TFIID binds to the major or minor groove of DNA (11).

We are investigating the conformation of I:C base pairs in crystal structures of deoxyoligonucleotides, and have previously reported the structure of d(CGCICICG) (12), which resembled the Z-DNA crystal structure of d(CGCGCG) (13). Other crystal structures containing inosine include mismatched I(*anti*):A(*syn*) base pairs in a B-DNA dodecamer (14), and I:T wobble base pairs in an A-DNA octanucleotide (15). In order to determine the conformation of I:C base pairs in B-DNA, the inosine-containing dodecamer, d(CGCIAATTCGCG), was designed based on the structure of d(CGCGAATTCGCG) (16; 17). We describe here the crystal structure of d(CGCIAATTCGCG), and its comparison with other B-DNA structures.

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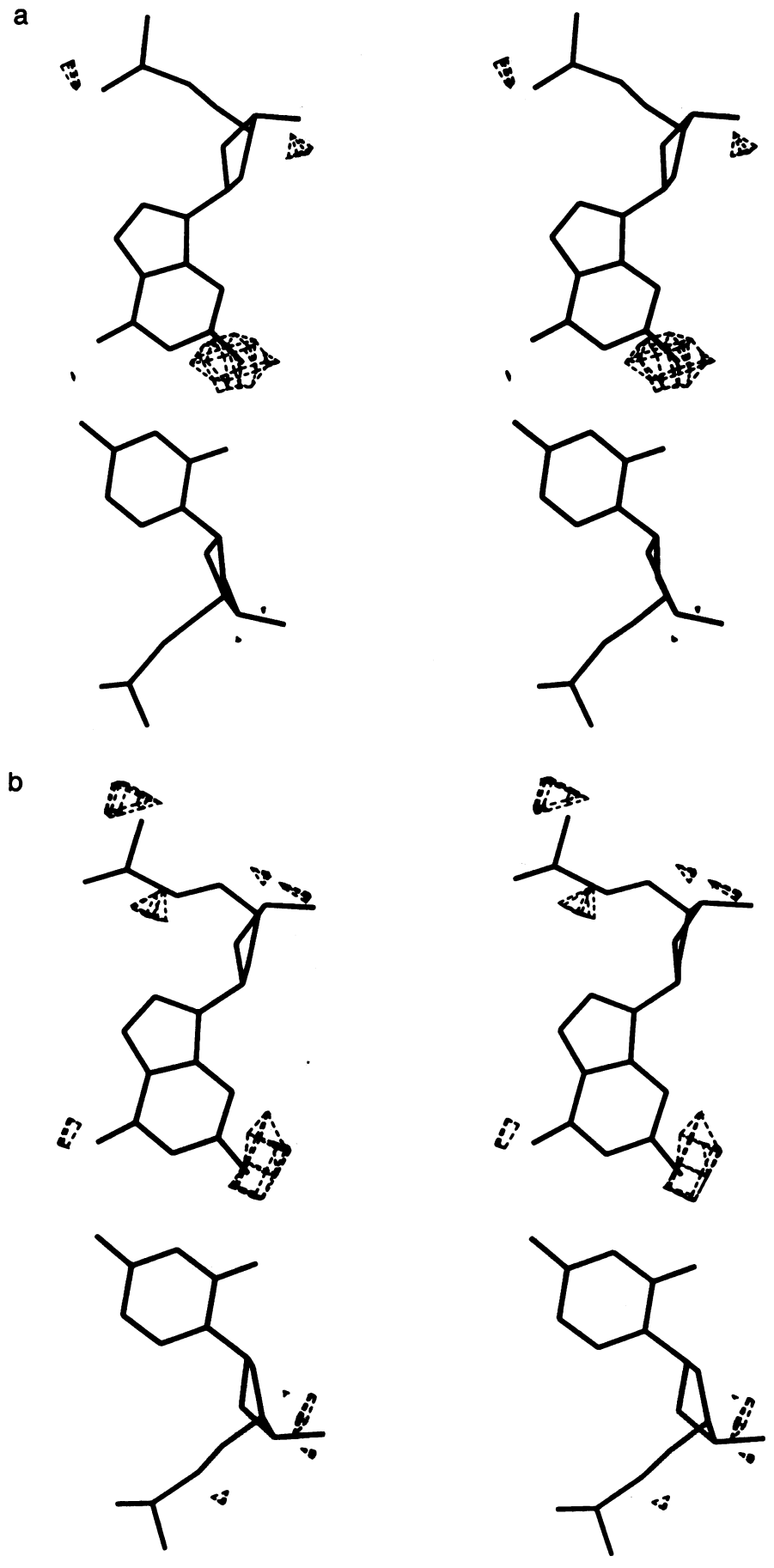
a
b

EXPERIMENTAL METHODS

The oligonucleotide, d(CGCIAATTCGCG), was synthesized by solid-phase phosphoramidite chemistry on an automated Applied Biosystems synthesizer, purified by reverse phase HPLC, and assayed by polyacrylamide gel electrophoresis. Crystals were grown at 4°C from a solution containing 1.2 to 1.3 mg/ml DNA, 8 mM magnesium acetate, 0.7 mM spermine and 12.5% methylpentanediol (MPD) by the vapor diffusion method. The drops were equilibriated against a reservoir containing 25% MPD. One rod-shaped crystal of size $0.1\times0.2\times0.7$ mm^3 was used for data collection. X-ray diffraction intensity data were recorded to 2.4 Å resolution at room temperature using a Siemens area detector mounted on a Rigaku RU-200 rotating anode generator, operated at 50kV and 100 mA. The space group was $P2_12_12_1$ with cell dimensions of a = 24.9, b = 40.4, and c = 66.4 Å. Two orientations of the crystal were measured and processed using XENGEN (18) to give 2492 unique reflections with a merging R value of 5.7%. The data are 99.7% complete from 20 to 2.5 Å, and 46.3% complete from 2.5 to 2.4 Å resolution.

The crystal structure was determined by molecular replacement using the isomorphous crystal structure of d(CGCGAATTCGCG) (17). Good 2Fo-Fc electron density maps were obtained after preliminary refinement against the coordinates of the parent DNA, d(CGCGAATTCGCG). The initial R-factor was 0.408, and dropped to 0.215 after 15 cycles of refinement with the program, NUCLSQ (19), using the d(CGCGAATTCGCG) coordinates with individual temperature factors and X-ray data from 8Å to 2.4Å resolution. The Watson–Crick base pair hydrogen bonds were restrained during refinement. The initial difference Fourier maps (Fo–Fc) are shown in Figure 1 for the two inosine-cytosine base pairs. The negative difference density at the 2-NH_2 group clearly indicated the presence of inosine at positions 4 and 16 in the two strands of the dodecamer. The G4 and G16 were replaced by inosine at those positions, and the structure was refined to convergence for another 34 cycles. At several stages of refinement, difference Fourier maps were examined on the PS300 Evans and Sutherland computer graphics system using the program, FRODO (20). Peaks in the difference maps representing solvent molecules were included as water. A conservative 44 water molecules were included in the final refinement. The final R-factor was 0.174, and the refinement statistics are shown in Table 1. The coordinates have been deposited in the Brookhaven Protein Data Bank. The program, NEWHEL91, provided by Richard Dickerson, was used to calculate the helical parameters and torsion angles. In order for NEWHEL91 to run correctly, I4 and I16 were renamed G4 and G16.

RESULTS AND DISCUSSION

The inosine-containing d(CGCIAATTCGCG) is in a B-DNA conformation similar to the parent dodecamer structure (Figure 2). The average B value for all atoms in d(CGCIAATTCGCG) was 15.7Å^2, for base atoms B=10.6, for ribose-phosphate atoms B=21.1, and for atoms P, O1P, and O2P B=26.1Å^2. As found previously, the ribose-phosphate atoms have higher B values than the atoms of the bases. C13 has the highest average B factor of 37.6Å^2, while the inosines have relatively low B-factors of 11.6 and 12.0Å^2 respectively, for I4 and I16. Overall the two dodecamer structures are very similar with root mean square (rms) deviation of 0.38Å for all atoms, 0.42Å for ribose-phosphate atoms, and 0.34Å for the atoms of the bases. Regions that are unrestrained by contacts to symmetry related molecules show larger rms deviations, not unexpectedly. These include the ribose-phosphate atoms of I4 to A6, A18 to G22, and the central base pairs from A5:T20 to T8:A17. The base pairs C1:G24 to I/G4:C21, and G10:C15 to G12:C13 show smaller deviations and are partly restrained by intermolecular contacts. A conservative number of solvent water molecules (44 with an average atomic B value of 34.8Å^2) were included in the refinement, compared to 80 for the parent structure (21). Sixteen water molecules (36.4%) in the structure of d(CGCIAATTCGCG) were within 1.25Å of the nearest water position in the parent dodecamer crystals, and 6 more (13.6%) were within 2.0Å. This appears to be relatively good agreement for the water structure in the two dodecamers.

Base pair geometry of I:C

The presence of the inosine was clearly indicated in the initial Fo–Fc difference maps by the negative density at the position of the 2-amino group that is present in G but not in I (Figure 1). The hypoxanthine base has replaced guanine and formed two I:C base pairs of a standard Watson–Crick type at positions I4:C21 and I16:C9. The lengths of the Watson–Crick hydrogen bonds are very close to those observed for the G:C base pairs in the parent DNA: 2.77Å between N1 of I4 and N3 of C21 and 2.75Å between O6 of I4 and N4 of C21, compared to 2.72 and 2.61Å between the same atoms in G4:C21. The corresponding distances for I16:C9 are 2.92 and 2.71Å compared to 2.81 and 2.72Å for G16:C9. No significant shear was observed for either I:C base pair, unlike the dodecamer structure with O6ethylG at positions 4 and 16 (22), which showed bifurcated hydrogen bonds for the O6ethylG4:C21 base pair and a wobble configuration for the O6ethylG16:C9 base pair, with hydrogen bond distances ranging from 2.60 to 3.16Å. The distance between N1 of inosine and O2 of cytidine was 3.50 and 3.95Å for I4:C21 and I16:C9 respectively, a slight increase over the 3.42 and 3.71Å observed for the equivalent G:C base pairs in the parent DNA, suggesting that there is no significant wobble interaction. Similar results were obtained for I:C base pairs in the Z-DNA structure of d(CGCICICG) (12); due to disorder in the crystal lattice, the I:C and G:C were indistinguishable. In contrast, non-Watson–Crick I(*anti*):A(*syn*) mismatched base pairs were observed in the crystal structure of d(CGCIAATTAGCG) (14), while wobble base pairing of I:T was observed in an A-DNA octanucleotide structure (15). This indicates that inosine can adopt a variety of base-pair configurations.

Figure 1. Stereo view of the I:C base pairs in the difference electron density between the Fo's for d(CGCIAATTCGCG) and the Fc's from the structure of the parent dodecamer, d(CGCGAATTCGCG), contoured at at level of $-2\ \sigma$. The negative difference density at the 2-NH_2 position indicated the replacement of guanine by inosine at positions 4 and 16. a) Base pair I4:C21; and b) base pair I16:C9.

Table 1. Refined statistics

R-factor	0.17	
Weights	$w = \sigma_F^{-2}$	
with	$\sigma_F = (0.8) + (-2.4)*(s-1/6)$	
Resolution range (Å)	8.0–2.4	
# of observations	2256	
# of atoms	528	
r.m.s. deviations from ideality (target restraints in parentheses)		
Distance restraints		
Sugar/base bond distances	0.012	(0.025) Å
Sugar/base angle distances	0.043	(0.050) Å
Phosphate bond distances	0.046	(0.050) Å
Phosphate bond angle distances	0.101	(0.075) Å
Plane restraiants	0.018	(0.030) Å
Chiral center restraints	0.077	(0.100) Å^3
Non-bonded restraints	0.161	(0.063) Å
Torsion angle restraints		
Omega,Psi	2.1	(2.0) (degree)
Phi,Chi	16.7	(15.0) (degree)
Psi',Phi',Omega'	27.1	(35.0) (degree)
B_{iso} restraints		
Sugar-base bonds	4.631	(7.5) Å^2
Sugar-base angles	5.392	(7.5) Å^2
Phosphate bonds	5.814	(7.5) Å^2
Phosphate bond angles	9.411	(7.5) Å^2

Local variation in helical parameters

The local helical geometry of d(CGCIAATTCGCG) was analysed using the program, NEWHEL91, and selected parameters are compared for d(CGCIAATTCGCG) and the parent dodecamer in Table 2. Most of the helical parameters are very similar, and follow the same trend for consecutive base pairs. The x-displacement values are a little larger for the inosine-containing dodecamer, with an average value of 0.23Å, compared to 0.05Å for d(CGCGAATTCGCG). The buckle angles show a larger variation for the I:C base pairs and for the adjacent base pairs. The propeller twist angles are larger for the central base pairs of the inosine-containing DNA from A5:T20 to C9:I16.

The two I:C base pairs have unexpectedly different propeller twist angles: I4:C21 has a low propeller twist angle of −3.18°, while I16:C9 has a relatively high value of −23.00° (Table 2). Both show larger buckle angles of −12.0° and 12.3°, for I4:C21 and I16:C9 respectively, compared to −8.2° and 9.6° for the equivalent G:C base pairs in the parent dodecamer. In fact, the largest change in buckle occurs at the next base pairs, with a change of −6.0° and −7.9° for A5:T20 and G10:C15, respectively. The larger propeller twist value for the I16:C9 pair is correlated with larger χ values by 24° and 20° for the glycosidic angles, and δ angles about 10° larger than in the parent

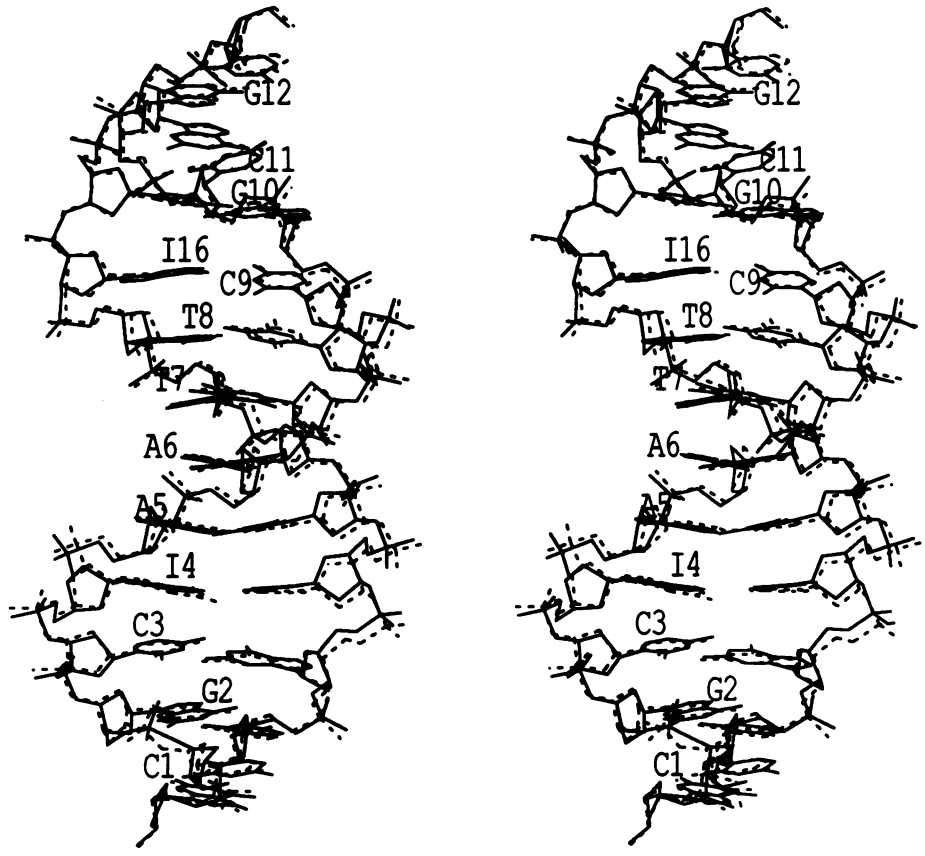

Figure 2. Stereo view of the crystal structure of d(CGCIAATTCGCG) in continuous lines, compared to the parent dodecamer structure of d(CGCGAATTCGCG) in dashed lines. Bases C1 to G12 and I16 are labeled.

dodecamer, while the glycosidic angles of I4 and C21 are within 3° of the corresponding angles in d(CGCGAATTCGCG).

The I4:C21 and I16:C9 base pairs lie at the junction between the central A:T region and the flanking G:C region, where there is a change in the direction of the base pair buckle. There are also different crystal packing interactions with G12# and G24#, where # indicates a symmetry related molecule (Figure 3). In the parent DNA, the O3′ of G12# forms an intermolecular hydrogen bond interaction with the 2-amino group of G22, and longer/weaker hydrogen bonds with the O2 of C23 and the

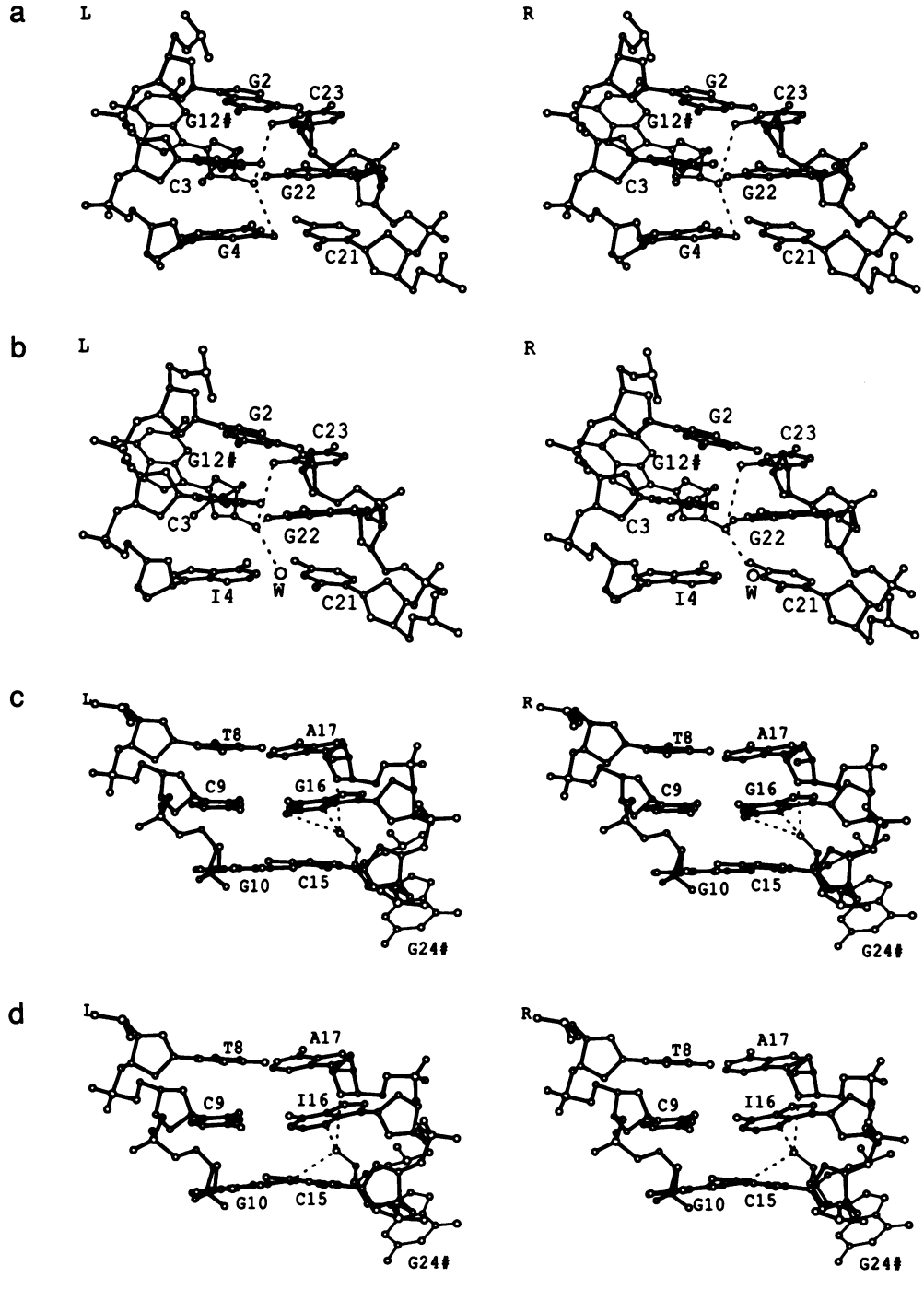

Figure 3. Stereoview of the intermolecular interactions. a) Base pairs G2:C23 to G4:C21 and G12# in the parent dodecamer, d(CGCGAATTCGCG); b) G2:C23 to I4:C21 and G12# in the inosine-containing d(CGCIAATTCGCG); c) Base pairs T8:A17 to G10:C15 and G24# in the parent dodecamer; and d) T8:A17 to G10:C15 and G24# in the inosine-containing DNA. The crystal structure is shown in thick lines and the symmetry related molecule is in thin lines and labeled #. Intermolecular hydrogen bond interactions are indicated by dotted lines, and W indicates a water molecule.

Table 2. Selected local helical parameters for comparison of the B-DNA structures of d(CGCIAATTCGCG) and d(CGCGAATTCGCG)

BASE PAIR	TIP	INCLINATION	ROLL	PROPELLER TWIST	BUCKLE	
C1:G24	5.0 (0.4)	10.3 (10.2)	−8.0 (1.0)	−16.3 (−13.0)	−8.3 (−1.6)	
G2:C23	−2.9 (1.4)	8.8 (9.0)	−6.9 (−9.2)	−15.4 (−10.8)	3.3 (4.4)	
C3:G22	−9.7 (−7.7)	7.8 (7.1)	6.3 (2.5)	− 5.6 (−3.7)	9.3 (6.1)	
I4:C21	−3.6 (−5.2)	3.6 (4.7)	2.8 (4.1)	− 3.2 (−10.4)	−12.0 (−8.2)	
A5:T20	−0.7 (−1.2)	0.9 (1.5)	−2.1 (0.5)	−17.1 (−16.2)	−11.5 (−5.5)	
A6:T19	−2.8 (−0.7)	−1.7 (−0.1)	−0.9 (−6.1)	−19.3 (−17.5)	−6.8 (−3.2)	
T7:A18	−3.7 (−6.8)	−0.8 (−0.6)	0.6 (1.1)	−19.8 (−17.0)	0.7 (−2.0)	
T8:A17	−3.1 (−5.6)	−2.7 (−1.9)	4.8 (5.0)	−19.7 (−17.1)	0.6 (0.2)	
C9:I16	1.7 (−0.6)	−3.2 (−4.0)	−0.1 (3.8)	−23.0 (−16.0)	12.3 (9.6)	
G10:C15	1.7 (3.2)	−3.1 (−3.8)	−4.5 (−6.5)	−9.2 (−4.7)	−9.5 (−1.6)	
C11:G14	−2.9 (−3.3)	−6.5 (−6.6)	7.4 (3.3)	−22.1 (−16.9)	−1.0 (3.1)	
G12:C13	4.5 (−0.0)	−5.9 (−6.9)	−	1.4 (2.4)	−8.9 (−5.7)	
	−1.4 (−2.2)	0.6 (0.7)	−0.0 (−0.0)	−14.1 (−11.8)	−2.7 (−0.4)	AV
	4.1 (3.5)	5.7 (5.9)	5.1 (4.9)	8.0 (6.6)	8.1 (5.3)	SD

Local helical parameters for the inosine-containing dodecamer compared to the values for the parent dodecamer in parentheses. Values were calculated for the best plane through both bases for tip, inclination, roll, propeller twist, and buckle angles using the program, NEWHEL91. The average value and standard deviation are also listed.

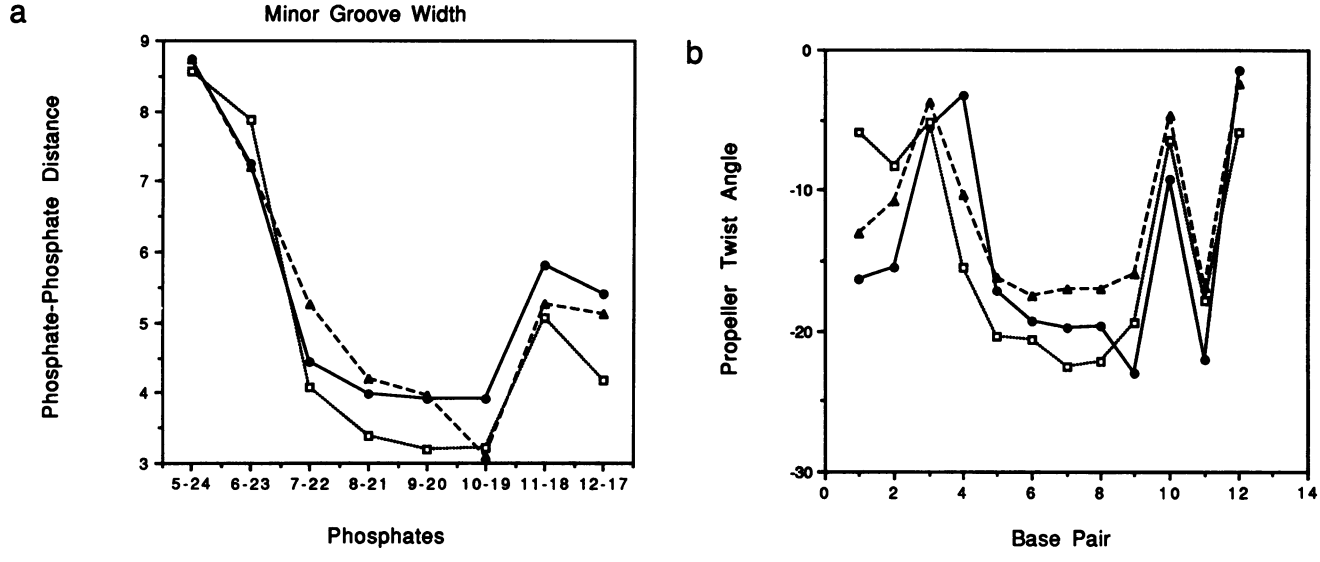

Figure 4. a) Minor groove Phosphate-Phosphate distances are compared for the inosine-containing d(CGCIAATTCGCG) (solid circles connected by continuous lines); d(CGCGAATTCGCG) (solid triangles connected by dashes); and the A-tract containing d(CGCAAAAATGCG) (open squares connected by dots). b) Propeller twist angles are compared for the inosine-containing d(CGCIAATTCGCG); the parent dodecamer, d(CGCGAATTCGCG); and the A-tract containing d(CGCAAAAATGCG), as indicated in 4a.

2-amino of G4, the base pairs above and below G22 (Figure 3a). The interaction of G4 with G12 # O3′ will tend to increase the propeller twist angle and −10.4° is observed. In the inosine-containing DNA, I4 lacks the 2-amino group, so G12 # O3′ forms a hydrogen bond interaction with a water molecule instead (Figure 3b). I4:C21 has lost the intermolecular interaction, the propeller twist angle is −3.2°, and the buckle changes to −12.0° compared to −8.2° in the parent DNA. There is a large change in buckle for the adjacent A5:T20 probably due to purine stacking interactions with I4. At the other position, G16 in the parent DNA has hydrogen bond interactions of both N3 and the 2-amino group with G24 # O3′ (Figure 3c). However, I16 lacks the 2-amino group, so the O3′ of G24 # forms a weak hydrogen bond interaction (3.7Å) with the 2-amino group of G10 instead (Figure 3d). This interaction tends to increase the buckle of G10:C15 to −9.5° from the value of −1.6° in the parent dodecamer, and the propeller twist also increases (−9.2° compared to −4.7°). The I16:C9 base pair shows a smaller increase in buckle from 9.6° to 12.3°, and the propeller twist angle is increased from −16.0° to −22.1° (Table 2). This increase in propeller twist appears to be propagated by base stacking from I16:C9 through the central A:T base pairs. The crystal structure of a dodecamer containing O6ethylG also showed differences in propeller twist and buckle (22). Base pair O6ethylG16:C9 has high propeller twist angles of −21° and −24°, and buckle of 12° or 24°; while O6ethylG4:C21 has low propeller twist angles of 6° and 2°, and large buckle of −18° and −22° for the structures with two different drugs. In this case,

the unusual values could be explained by interactions with the drug, or the non-standard base pair configurations observed for the two O6ethylG:C base pairs.

The structure of d(CGCIAATTCGCG) resembles A-tract DNA

The hypothesis is that the I:C base pairs will resemble A:T rather than G:C base pairs in the local helical parameters, since I:C base pairs form only 2 hydrogen bonds like A:T, rather than the 3 of G:C base pairs. Several B-DNA crystal structures containing A-tracts have been determined (23; 24; 25) and the local helical parameters have been compared previously (26; 27). The A:T regions have higher propeller twist angles, narrower minor grooves, and show cross-strand bifurcated (or three-centered) hydrogen bonds in the major groove compared to the G:C regions. In contrast, the two A-DNA crystal structures of the octamer d(GTGTACAC) showed higher propeller twist angles for G:C compared to A:T base pairs (28). Selected local helical parameters are compared in Table 2 and Figure 4 for the inosine containing dodecamer, d(CGCIAATTCGCG), the parent, d(CGCGAATTCGCG), and d(CGCAAAAATGCG) (25). The structure of d(CGCAAAAATGCG) is the longest A-tract dodecamer available in the Protein Data Base, and contains two disordered molecules, designated the up and down helices. The up helix values were used since these were more uniform than those for the down helix, and appear to be a relatively extreme example of A-tract helical parameters.

The inosine-containing dodecamer has a narrow minor groove similar to the parent dodecamer, but a little wider than observed for the longer A-tract containing structure of d(CGCAAAAATGCG) (Figure 4a). The propeller twist angles for the central base pairs are slightly larger than for the parent dodecamer and show changes at the two I:C base pairs, as described earlier (Figure 4b). The average value is $-14.1°$ for the 12 base pairs, similar to the values of $-14.2°$ and $-12.1°$ for the two molecules in the long A-tract structure of d(CGCAAAAATGCG), and the value of $-14.3°$ for the decamer d(CCAAGATTGG), where the central GA mismatch resulted in a larger propeller twist angle (29). Smaller average propeller twist angles of $-10.3°$ and $-11.8°$ respectively, were observed for d(CCAACGTTGG) (29), and the parent dodecamer (Table 2). The mean propeller twist for different B-DNA helices was reported to be in the range of $-17°$ to $-22°$ for A:T base pairs, compared to $-8°$ to $-14°$ for G:C base pairs (26). The larger values occurred in B-DNA with central A-tracts, or polyA regions. In the inosine-containing B-DNA, the mean values of propeller twist are $-19.0°$ for the 4 central A:T base pairs, which is larger than the value of $-17.0°$ observed for the parent dodecamer, and $-11.7°$ for the 6 G:C base pairs, also larger than the value of $10.2°$ for the parent dodecamer. The larger propeller twist values for the A:T base pairs in d(CGCIAATTCGCG) may be due to correlated changes for the stacked purines from I16:G9 to A18:T7.

Yanagi *et al.* (27) proposed that there were two requirements for formation of large propeller twist angles: 1) A:T rather than G:C base pairs; and 2) formation of major groove three-centered hydrogen bonds with the following base pair. The structure of inosine-containing d(CGCIAATTCGCG) showed the large propeller twist angles typical of A-tracts, although I:C cannot form the bifurcated hydrogen bonds of A:T base pairs, since inosine has a hydrogen bond acceptor 6-O rather than the hydrogen bond donor 6-NH2 of adenosine. This suggests that the formation of 3-center hydrogen bonds is not essential for large propeller twist angles, as was originally proposed by Nelson *et al.* (24) and Coll *et al.* (23).

CONCLUSIONS

The crystal structure of d(CGCIAATTCGCG) is a B-DNA dodecamer containing two I:C base pairs. The I4:C21 base pair has a smaller propeller twist angle of $-3.2°$, compared to the larger value of $-23.0°$ for the I16:C9 base pair, which is at least partly due to different intermolecular contacts. The central base-pairs have the large propeller twist angles and narrow minor groove characteristic of B-DNA containing A-tracts, although I:C base pairs cannot form the three-centered hydrogen bonds in the major groove that Yanagi *et al.* (27) proposed were required for the formation of large propeller twist angles. Both this structure, and the Z-DNA structure of d(CGCICICG) (12), show I:C base pairs in a similar conformation to the G:C base pairs. Inosine can be substituted for guanosine in both B- and Z-DNA conformations, and forms Watson–Crick type I:C base pairs. In contrast, inosine substitutions for thymidine or adenosine, have resulted in the non-Watson–Crick I(*anti*):A(*syn*) mismatch (14) and an I:T wobble base pair (15). The conformation of the base pair will depend on both the sequence of adjacent bases and the local environment in the crystal.

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