Crystal and Molecular Structure of a New Z-DNA Crystal Form: d[CGT(2-NH_2-A)CG] and Its Platinated Derivative[†,‡]

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ABSTRACT: The three-dimensional structure of d[CGTA′CG], where A′= [2-NH_2-A], was determined to atomic (1.35 Å) resolution by single isomorphous replacement. The d[CGTA′CG] hexamer crystallizes in space group $P3_221$, and is not isomorphous with other DNA hexanucleotides. Despite completely different crystal packing, the essential characterisitics of the Z-DNA conformation are maintained. The structure was determined by single isomorphous replacement using a triammine platinum fragment. Thus, this study also demonstrates, for the first time, the feasibility of the use of this reagent for the direct phasing of DNA crystal structures.

DNA hexamer sequences of alternating CG bases crystallize as left-handed helices with the Z-conformation. This conformation has also been found for sequences containing UA (Geierstanger et al., 1991), TA (Wang et al., 1984), AT (Wang et al., 1985), or UA′ (Schneider et al., 1992) in the central step. The structure reported herein is of a short, modified DNA strand of sequence d[CGTA′CG], where A′ = [2-NH_2-A]. This modified base that was found to substitute for adenine in at least one organism (Kirnos et al., 1977) was introduced into this sequence because it was thought that the third hydrogen bond donated by the 2-amino group allows A′-T to mimic the C-G hydrogen bonding geometry (Singer & Kusmierek, 1982). Thus, it appeared likely that the sequence would maintain the Z-DNA conformation.

The crystals reported in this study are in space group $P3_221$. [It has been reported that this sequence crystallizes in a hexagonal lattice and was assumed to be disordered (Coll et al., 1986), but this is not the case.] As a consequence, the packing of the helices in the crystal must be completely different. Since it can now be demonstrated conclusively that Z-DNA can exist in a different crystalline environment, this study emphasizes that the sequence itself has characteristics that favor this conformation.

Since d[CGTA′CG] is nonisomorphous with other DNA hexamer crystals, its structural conformation was not certain. When initial attempts at phasing through molecular replacement proved unsuccessful, isomorphous replacement was tried. This method has been used for very few DNA structures, notably $[d(CGCGCG)]_2$ (Wang et al., 1979) using three crystal soaked derivatives, and $[d(CGCGAATTCGCG)]_2$ (Drew et al., 1981), synthesized with 5-bromodeoxycytidine at position three, and one crystal soaked derivative. Rather than synthesizing a modified oligonucleotide, the use of a platinum-containing triammine species to form a complex was investigated. Surprisingly, structure determinations resulting from soaking DNA crystals with PtN_3X moieties have not been reported, although the high degree of site-specificity in the binding of platinum to guanine bases is well known (Chu, 1978). The monofunctional binding of platinum triammines to oligonucleotides has been studied by NMR (Van Garderen et al., 1988). Furthermore, the possibility of nondisruptive binding of a platinum triammine has been shown previously (Macquet & Butour, 1978a,b), making this metal an attractive candidate for the preparation of heavy atom derivatives. The demonstrated success of this reagent for this structure determination provides new possibilities for direct crystal structure determinations of new sequences of oligonucleotides.

In addition to its use as an aid in DNA crystal structure determination, there is also strong biochemical interest in the binding of platinum to nucleic acids, especially since some Pt reagents have antitumor activity (Hollis, 1989). The binding of $Pt(NH_3)_3$ to a DNA hexamer may provide further data to help sort out the puzzling and little understood relations among DNA binding, mutagenicity, and effective antitumor activity in Pt-containing species.

MATERIALS AND METHODS

Purified and lyophilized d[CGTA′CG] was prepared as described previously (Gaffney et al., 1984). Crystals were grown by vapor diffusion in sitting drops at room temperature. The initial drop solution contained 10% MPD (2-methyl-2,4-pentanediol), 10 mM spermine, 15 mM $MgCl_2$, 30 mM sodium cacodylate, and 2mM d[CGTA′CG] and was equilibrated against a 20% MPD well solution. The crystals grew as rectangular blocks 0.8 × 0.4 × 0.3 mm in 20 μL of drop solution.

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[‡] The coordinates have been deposited in the Brookhaven Protein Data Bank (file names 210D and 211D).

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Table 1: Crystal Data and Collection Statistics for Both Native and Derivative Data Sets

	native	platinated
	Crystal Data	
contents of asymmetric unit		
molecules of d[CGTA′CG]	1	1
formula	$C_{58}H_{70}N_{24}O_{34}P_5$	$C_{58}H_{70}N_{24}O_{34}P_5Pt(NH_3)_2$
molecular weight	1802.21	1876.05
space group	$P3_221$	$P3_221$
unit cell		
$a = b =$ (Å)	25.247(6)	25.193(3)
$c =$ (Å)	39.14(2)	38.948(6)
	$\alpha = \beta = 90°, \gamma = 120°$	$\alpha = \beta = 90°, \gamma = 120°$
volume (Å^3)	21 651	21 407
density		
D_{calc} g cm^{-3}	1.45	not measured
D_{meas} g cm^{-3}	1.45 ± 2	not measured
	Data Collection Statistics	
crystal size (mm)	0.9 × 0.4 × 0.14	0.4 × 0.2 × 0.12
temperature	18 °C	4 °C
crystal mounting	capillary	capillary
data collection device	Enraf-Nonius CAD4 rotating anode	Enraf-Nonius CAD4 rotating anode
radiation	Cu Kα ($\lambda = 1.54178$)	Cu Kα ($\lambda = 1.54178$)
unique data collected	4487	2129
reflections $F > 2\sigma(F)$	3408	1741
mean fractional isomorphous difference on $\|F\|$		0.19
resolution limit (Å)	1.35 Å	1.60 Å
% reflections > $4\sigma(F)$	up to 2.10 Å 94	up to 2.50 Å 92
	2.10−1.50 Å 76	2.5−1.95 Å 74
	1.50−1.35 Å 53	1.95−1.60 Å 60

Among the platinum compounds available for complexing to the native crystal structure were *cis*-[$(NH_3)_2$(4-BrC_5H_4N)ClPt]NO_3 (Hollis et al., 1989), [$(NH_3)_3$ClPt]Cl (Morita & Bailar, 1983), *trans*-[$(NH_3)_2$(1-methylimidazole)-ClPt]NO_3 (Gibson et al., 1994), and [(diethylenetriamine)-PtCl]Cl (Watt, 1968). Kinetic studies were initially performed on d[CGTA′CG] using *cis*-[$(NH_3)_2$(4-BrC_5H_4N)-ClPt]NO_3 to determine the reactivities of the possible binding sites to platinum compounds. Crystal soaking experiments were tried using both *cis*-[(4-BrC_5H_4N)$(NH_3)_2$ClPt]NO_3 and [$(NH_3)_3$ClPt]Cl. Soaks using *cis*-[(4-BrC_5H_4N)$(NH_3)_2$ClPt]-NO_3 resulted in cracked, fragile crystals. After several initial attempts, a crystal soaked for 3 weeks with a 4.25 molar excess of [$(NH_3)_3$ClPt]Cl gave large observable differences in intensities between native and derivative data sets without modifications in the cell dimensions.

Both native and derivative crystals were mounted in thin-walled capillaries with mother liquor at base and top. Unit cell dimensions were determined from a least-squares treatment of 25 high-angle reflections. Measurements were made on an Enraf-Nonius CAD4 diffractometer with a rotating copper anode ($\lambda = 1.54178$ Å) and graphite monochromator. Systematic absences and symmetry-equivalent reflections from several native crystals indicated two possible trigonal space groups, the enantiomorphic pair $P3_221$ or $P3_121$. Packing and crystal density constraints predicted one d[CGTA′CG] strand in the asymmetric unit with approximately 43% by weight solvent and cations.

The native data were collected to a resolution of 1.35 Å at 18 °C; the heavy atom derivative data were collected to a resolution of 1.60 Å at 4 °C (Table 1). Data reduction on all data sets was carried out using MoLEN (Fair, 1990). Three reflections were used to monitor decay and apply scaling. Absorption corrections were made based on ψ-scan intensity variations. Full intensity profiles were collected and integrated using the Lehmann and Larson method (Lehmann & Larsen, 1974). The program PHASES (Furey, 1985) was used for merging data sets and for heavy atom refinement, solvent flattening (Wang et al., 1985), and phase refinement. Native and derivative data sets were each merged using Wilson scaling.

Table 2: Refined Heavy Atom Positions and Phase Determination Statistics

x	y	z	B(Å^2)	occupancy	RF[a]
0.684	0.762	0.331	15	0.30	43%

resolution limit (Å)	7.30	4.10	3.55	3.15	2.86	2.64	2.47	2.33	2.22	2.12
phasing power[b]	3.08	2.46	2.24	1.85	2.16	2.08	2.07	1.95	1.81	1.75
mean figure of merit[c]	0.73	0.64	0.62	0.48	0.46	0.38	0.36	0.28	0.27	0.21

[a] RF = $\sum||F_o| - |F_c||/\sum|F_o|$. [b] Phasing power =

$$\left[\frac{\sum_n |F_H|^2}{\sum_n |E|^2}\right]^{1/2}$$

with $\sum_n |E|^2 = \sum_n \{|F_{pH}|(obs) - |F_{PH}|(calc)\}^2$ where $|F_H|$ = is the calculated heavy atom structure factor amplitudes, and n = is the number of observed scattering amplitudes for the derivative. [c] Mean figure of merit = $|F(hkl)_{best}|/|F(hkl)|$, where $F(hkl)_{best} = \sum_\alpha P(\alpha)Fhkl(\alpha)/\sum_\alpha P(\alpha)$.

Isomorphous difference Patterson maps were calculated to locate heavy atom sites. The most consistent solution for the observed Patterson peaks was a heavy atom at $x = 0.684$, $y = 0.762$, $z = 0.331$ or the 3_2 related $x = 0.684$, $y = 0.762$, $z = 0.833$. Refinement of the heavy atom position was carried out with more than 75% of the centric data. A platinum atom at $x = 0.68$, $y = 0.76$, $z = 0.33$ was used for the initial phase determination based on 2 Å data. The overall phasing power (defined in Table 2) was 2.18; the

Table 3: Refinement Statistics for Both the Native and Derivative Data Sets

	native	platinated
resolution (Å)	10−1.35 Å	12−1.60 Å
reflections used ($\|F_o\| > 4\sigma$)	2562	1340
number of water positions included in refinement	24	24
residual value = $\sum\|\|F_o\| - \|F_c\|/\sum\|F_o\|$	17.4	17.0
residual electron density (e/Å^3)max/min	0.47, −0.355	0.29, −0.30
deviations from ideal values (Parkinson et. al., 1995)		
bonds (Å)	0.007	0.009
angles (deg)	1.88	1.66
dihedrals (deg)	9.2	10.4
impropers (deg)	1.12	1.24

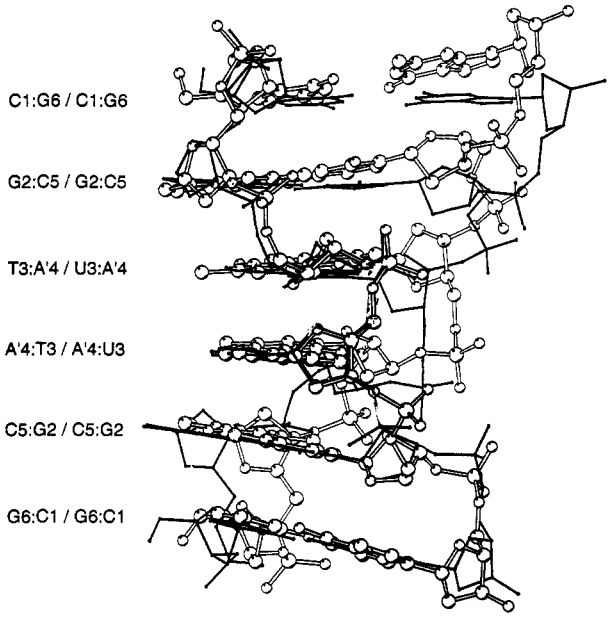

FIGURE 1: Structure of d[CGTA'CG]$_2$ shown as ball and stick compared with d[CGUA'CG]$_2$ shown as line bonds. The nomenclature for the bases are shown on the left side of the diagram.

correlation coefficient was 0.877.

Heavy atom structure factors were calculated and used to estimate phases for the native data. A solvent mask was generated using standard parameter values. Solvent flattening using negative density truncation was used to refine the initial phases derived from the platinum atom and resulted in a correlation coefficient of 0.931, a mean figure of merit of 0.767, and an $R = 31\%$ for 838 reflections. Fourier maps calculated from these initial estimates of native phases showed the DNA bases and phosphate groups unambiguously. The best fit was to a left-handed Z-DNA hexamer in space group $P3_221$. Table 2 gives the phase refinement statistics.

The native structure model was initially improved with constrained and restrained least-squares refinement using SHELXL-93 (Sheldrick, 1994). Electron density fitting was carried out using CHAIN 5.0 (Sack, 1988). Water molecules were included if electron density above 2.5σ was observed and if there was corresponding density on the $2F_o - F_c$ electron density maps. A long chain of residual density was observed along the phosphate backbone and was modeled as a spermine molecule. Its occupancy refined to 40%. The latter stages of refinement were done using X-PLOR (Brünger et al., 1987) using a new parameter file for the

Table 4: (a) The RMS Deviations Calculated between the Single Strand of d[CGTA'CG] in the ZI and ZII Conformations and the Single Strands in the [d(CGCGCG)]$_2$ and [d(CGUA'CG)]$_2$ Duplexes, and (b) The RMS Deviations between [d(CGTA'CG)]$_2$ and Duplexes of [d(CGCGCG)]$_2$ and [d(CGUA'CG)]$_2$

(a)	d[CGCGCG] ZI strand	d[CGCGCG] ZII strand	d[CGUA'CG] ZI strand	d[CGUA'CG] ZII strand
d[CGTA'CG] ZI	1.1 Å		0.9 Å	
d[CGTA'CG] ZII		0.8 Å		0.5 Å

(b)	[d(CGCGCG)]$_2$	[d(CGUA'CG)]$_2$
[d(CGTA'CG)]$_2$	1.0 Å	1.0 Å

Table 5: Key Base Morphology Parameters (Babcock & Olson, 1992) of d[CGTA'CG] as Compared to [d(CGCGCG)]$_2$ (Gessner et al., 1989)

	tilt		twist		roll	
base pair steps	TA'	CG	TA'	CG	TA'	CG
1/2	0.7	−0.6	12.8	7.7	−5.3	−2.7
2/3	−2.2	0.2	41.7	50.1	−9.8	−7.4
3/4'	0.0	0.6	6.7	7.7	−4.6	−2.2
4'/5	2.2	−1.0	41.7	51.3	−9.8	−2.8
5/6	−0.7	−1.0	12.8	9.8	−5.3	−0.9

	buckle		propeller		open	
base pairs	TA'	CG	TA'	CG	TA'	CG
1	1.8	−1.0	−4.0	−0.6	7.4	4.1
2	3.6	7.1	−2.3	−3.0	6.6	4.0
3	−9.5	−4.8	−1.7	−7.3	0.6	4.4
4	9.5	9.0	−1.7	0.6	0.6	2.9
5	−3.6	−0.3	−2.3	−0.3	6.6	2.5
6	−1.8	−5.6	−4.0	4.9	7.4	2.3

DNA (Parkinson et al., 1995). The final residual for the native structure is $R = 17.4\%$ and the Free $R = 23.0\%$ (Hodel et al., 1992). Refinement statistics are shown in Table 3. Coordinates and structure factors have been deposited with the Protein Data Bank (210D).

The refined native DNA structure was then used as the phasing model for the platinated structure. Difference Fourier maps showed strong density for a platinum atom located in the major groove, 2.18 Å from N7 of guanine-6. This was known to be the preferred binding site for platinum amines. The second possible platinum binding site at N7 of guanine-2 was unavailable due to intermolecular contacts between the bases and the DNA backbone.

The platinated d[CGTA'CG] structure model was improved with constrained and restrained least-squares refinement using SHELXL-93. The platinum atom refined to an occupancy of 30%. Difference Fourier maps in the latter stages of refinement showed residual density at three sites approximately 2.0 Å from Pt indicating the amines. The $(NH_3)_3$ geometry around the platinum atom was restrained using values obtained from *trans*-[$(NH_3)_2$(N7-guanosine)-ClPt]NO_3 (Arvanitis et al., 1994). The final refinement cycles were done with X-PLOR. The final R factor is 17.0%. Refinement statistics are shown in Table 3. Coordinates and structure factors have been deposited with the Protein Data Bank (211D).

RESULTS

Native DNA Structure. The DNA sequence d[CGTA'CG] crystallizes as a left-handed Z-DNA duplex (Figure 1). The

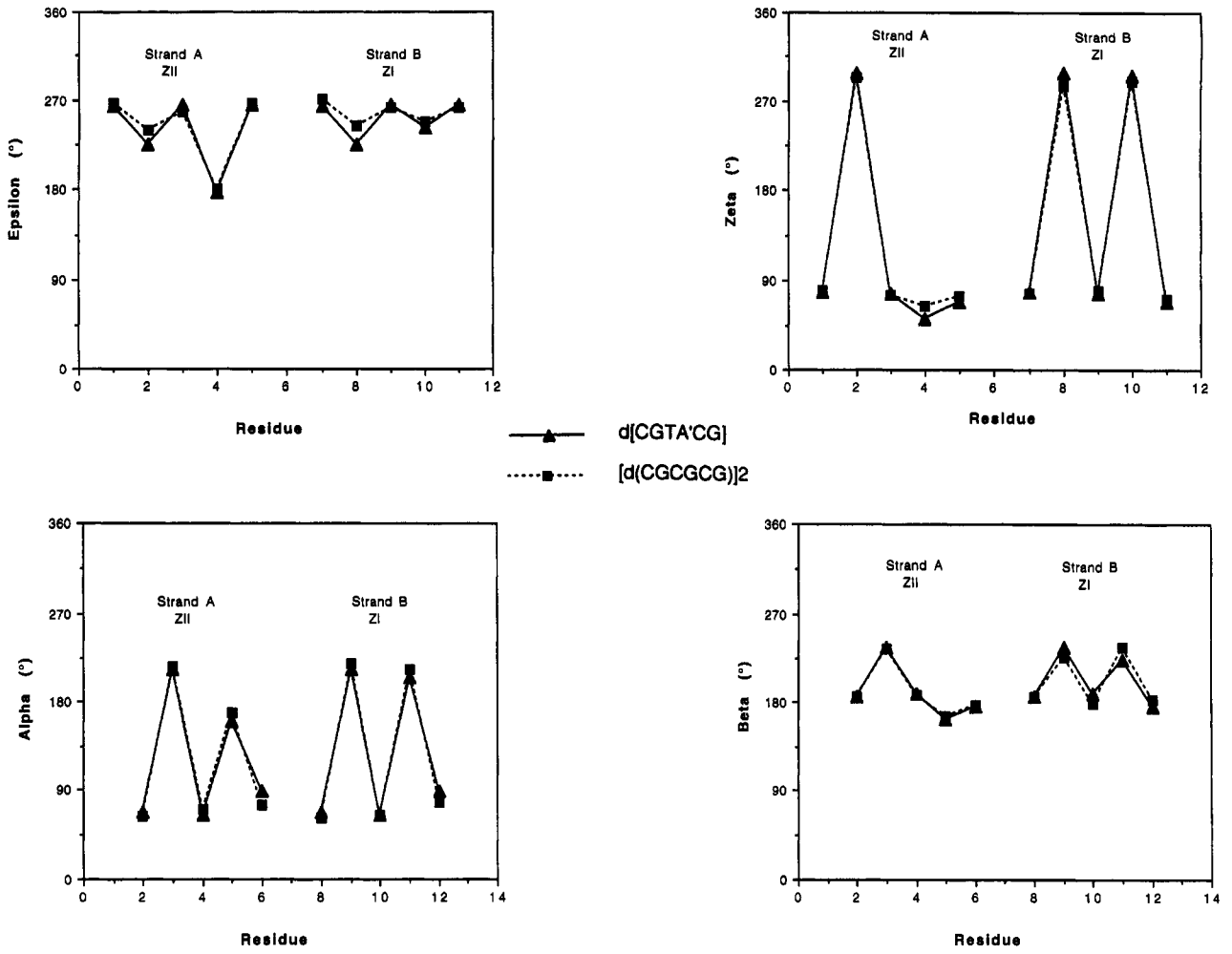

FIGURE 2: Comparison of the torsion angles for the ZI and ZII backbone conformations of both d[CGTA'CG] and $[d(CGCGCG)]_2$ (Gessner et al., 1989). The torsion angles for d[CGTA'CG] are shown as triangles. The ZI conformation is shown on the right side of each diagram and the ZII conformation is shown on the left. The values for strand A and strand B of $[d(CGCGCG)]_2$ are shown as squares.

root mean square differences between d[CGTA'CG] and $[d(CGCGCG)]_2$ (Gessner et al., 1989) as well as with its closest relative $[d(CGUA'CG)]_2$ (Figure 1) (Schneider et al., 1992) range from 0.5 to 1.1 Å for each DNA strand (Table 4). The rms differences between the duplexes are both about 1 Å.

The overall differences between the DNA strands and duplexes are reflected in significant differences between some of the calculated helical parameters (Babcock & Olson, 1992) of this structure and $[d(CGCGCG)]_2$ (Table 5). Z-DNA structures always display roll into the minor groove. In this duplex, the roll is enhanced for all the base steps. This results in a more narrow minor groove. The second and fourth steps display the highest negative roll. In addition, the twist is reduced by 9° at these steps. The duplex is unwound by 12° compared to $[d(CGCGCG)]_2$. Finally, it is interesting that the base pair between the T and A' has a smaller propeller twist than the normal CG base pair at that position.

All previously reported Z-DNA crystal structures contain two DNA strands in the asymmetric unit. For the majority of these structures, all the residues in one strand display a ZI conformation, in which the values for torsion angles of the pyrimidine and purine residues alternate (Wang et al., 1981). In the ZII conformation, this alternation is broken at steps 4 and 5 in one strand of the hexamer. The values for torsion angles involving the phosphodiester linkage, α, β, ε, and ζ are different. In the structure reported here, the asymmetric unit consists of a single strand of d[CGTA'CG]. The duplex is formed around the crystallographic two fold axis. Thus, in principle, the two strands must be identical. However, the electron density maps displayed diffuse electron density in the DNA backbone near the phosphodiester linkage at position C5. This density was fitted and refined with a model disordered between the two backbone geometries, ZI and ZII, with occupancies of 60% and 40%, respectively. Figure 2 and Table 6 compare the conformation angles in this structure with those found in $[d(CGCGCG)]_2$ (Gessner et al., 1989). The spermine molecule which also has 40% occupancy lies along the phosphate backbone of a symmetry-related molecule; its terminal nitrogen forms a

Table 6: Conformation Angles in d[CGTA'CG][a]

residue	α	β	γ	δ	ε	ζ	χ
C1			47	138	264	79	208
G2	68	186	174	95	225	298	67
T3	214	237	54	140	265	77	193
A'4	65	189	181	102	242/178[b]	296/51[b]	61
C5	205/160[b]	224/163[b]	62	142	265	69	206
G6	89	177	177	138			75

[a] Angles are defined in degrees as follows: α = O3'-P-O5'-C5'; β = P-O5'-C5'-C4'; γ = O5'-C5'-C4'-C3'; δ = C5'-C4'-C3'-O3'; ε = C4'-C3'-O3'-P; ζ = C3'-O3'-P-O5'; χ = O4'-C1'-N1py/N9pu-C2py/C4pu. [b] The ZI and ZII backbone conformations are provided for the disordered step at position four.

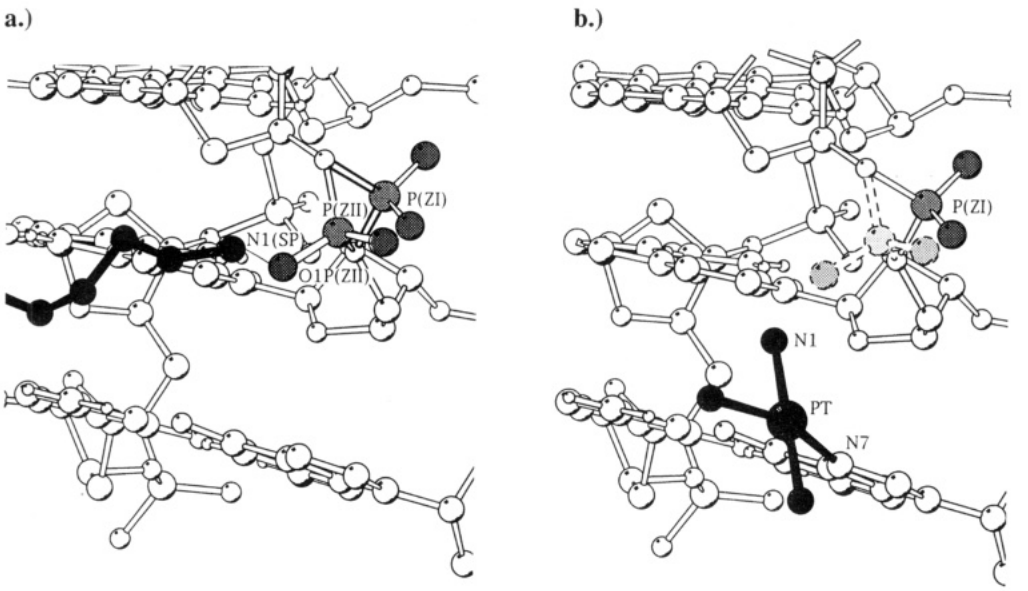

FIGURE 3: (a) ZI and ZII backbone conformations for C5 are shown. The N1 of the spermine is 2.5 Å from C5-O1P in the ZII conformation. (b) Binding of platinum in the crystal. In the platinated hexanucleotide strands, only the ZI conformation exists. The O1P in the ZII conformation would be 1.8 Å from the N1 of the platinum complex.

hydrogen bond of length 2.5 Å with the phosphate oxygen C5-O1P in the ZII conformation (Figure 3a).

The Platinum-Bound DNA Structure. The conformation of the DNA in the platinated crystal is very close to that seen in the native crystal. This was expected given the apparent isomorphism. The rms deviations between the six bases of bound and unbound forms is 0.3 Å. The water molecules in both the native and platinated hexamers are also close to one another; 20 of the 24 observed waters are within 0.4 Å of each other. Only those waters associated with the site of platinum binding are disrupted.

The relative ZI and ZII populations are different. The binding of the platinum would generate a sterically impossible contact between one of the platinum amine groups and the O1P phosphate oxygen in the ZII conformation. Thus, the presence of platinum is incompatible with the ZII conformation. As a result, the occupancy of the ZII is reduced in the crystal and the occupancy of ZI is increased in the platinated crystal. The lower population of the ZII conformation is coupled with the lower occupancy of the spermine in the platinated crystal. It is possible that the deterioration of the crystal after a long period of soaking may be related to the decrease in the population of ZII conformations.

The geometry about the square-planar platinum atom, including binding to the N7 of G6, is shown in Figure 3b. There are no significant differences in geometry of this guanine ligand and other platinated guanosines (Melanson & Rochon, 1978; Sherman et al., 1985, 1988). An amine ligand forms a bridge between platinum and O6 of the same guanine, which may serve to stabilize the complex (Goodgame et al., 1975). A similar interaction between a water ligand and O6 was found in the structure of cisplatin bound to $[d(CGCGAATTCGCG)]_2$ (Wing et al., 1984). Unlike the cisplatin-containing structure in which three guanines are platinum-substituted, only one guanine site is occupied in this case. Cisplatin binding to $[d(CGCGAATTCGCG)]_2$ is accompanied by a modest shift in the base pair stack, with G12 moving slightly out into the major groove. Such disruptions in the helix are suspected as important in the overall cytotoxicity of cisplatin. The binding of $Pt(NH_3)_3$ to d[CGTA′CG] shows no evidence of such a shift. The lack of movement of CG is most likely because Z-DNA is conformationally less flexible than B-DNA and not a result of any difference between platinum triammine and diammine.

Packing and Hydration. There are no significant differences between the native and platinum structures with respect to the packing arrangement. The overall packing arrangement of d[CGTA′CG] is shown in Figures 4 and 5. The terminal CG base pair of one duplex packs against the bases and backbone of the shallow major groove of a neighboring molecule near the central TA′ pair (Figures 4b). This contact is analogous to what has been observed in some forms of A-DNA, where terminal base pairs pack against the shallow minor groove of another duplex (Wang & Rich, 1987). In addition to these packing interactions, there are hydrogen-bonding or other short contacts between symmetry-related molecules (Figure 5b). The amide G6-N2 is 3.3 Å from nitrogen A′4-N7 on a second duplex. The G6-O3′ from one molecule accepts a hydrogen bond from the nitrogen G2-N7 of a third symmetry-related DNA strand. This particular hydrogen bond restricts the possibility of a second heavy atom ligand binding site at G2-N7. It is of interest to note that the terminal hydroxyl of the sugar G6 is involved in packing interactions observed in other Z-DNA structures bonding to a phosphate oxygen of the second base.

If waters involved in packing interaction are excluded, the hydration pattern for d[CGTA′CG] duplex is very similar to that found for $[d(CGUA′CG)]_2$; 10 of the 12 waters are within 1.5 Å (less than 2σ) of their counterparts, and the rms deviation is 1.0 Å. Six of these waters, located in the minor groove of d[CGTA′CG], form the characteristic spine of hydration. The most significant difference is found in the region of 2 and 3, where one expected water position was not occupied because the groove is narrowed too far in this region. The spine of hydration is shifted slightly within this local region to compensate and maintain the same overall acceptor−donor network.

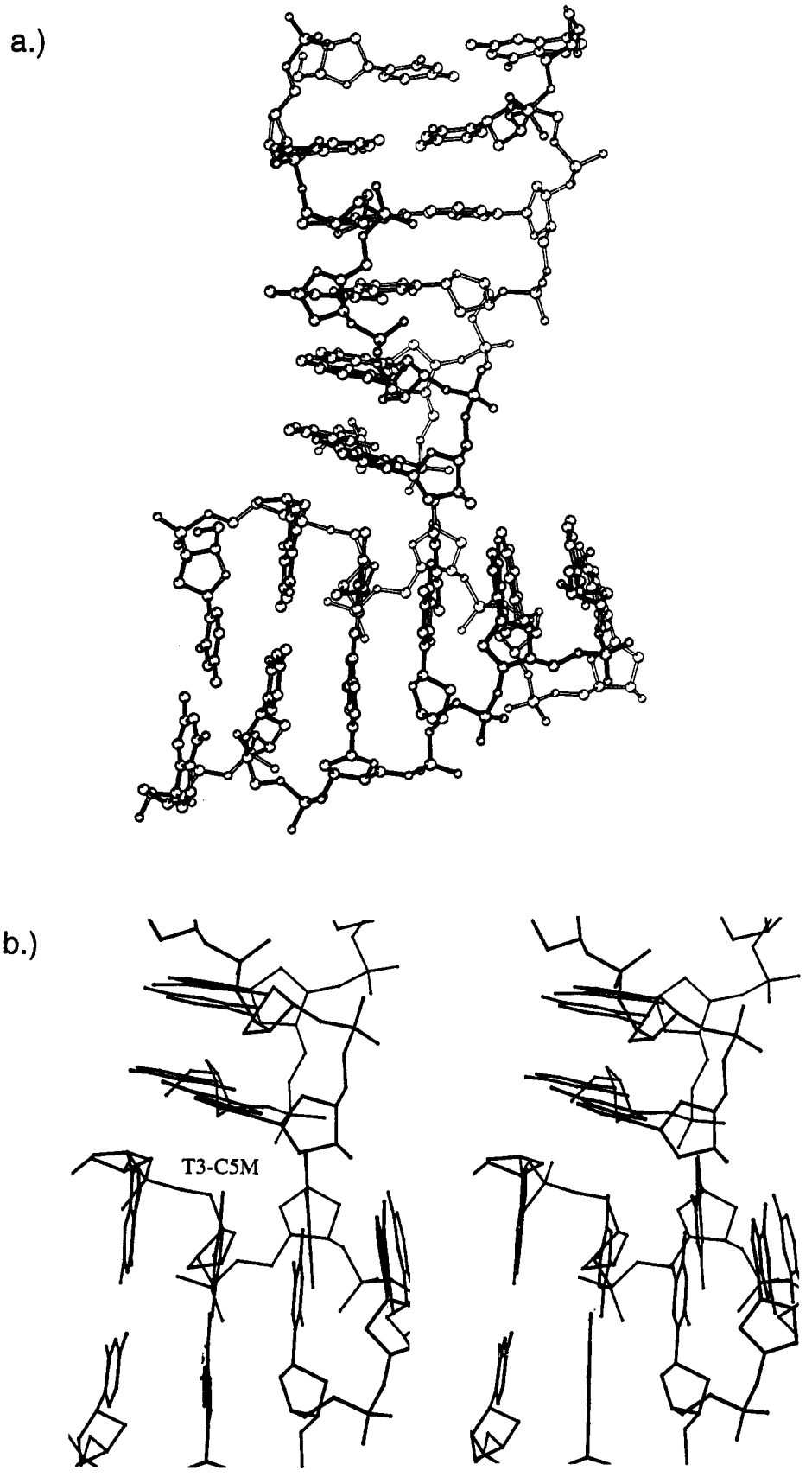

FIGURE 4: (a) Molecular packing diagram for d[CGTA′CG]$_2$ showing the interaction of the helices in the crystal. (b) Stereodiagram showing the interaction between the methyl group of T3 and G6 of the symmetry-related molecule.

DISCUSSION

The structure of a new crystal form of Z-DNA supplies information augmenting previous descriptions of the relationships among conformation, hydration, and other interactions. It again illustrates that the Z-DNA backbone is relatively inflexible. The molecule accommodates changes in crystalline form without significant alteration in its preference for the ZI-ZII conformational mix.

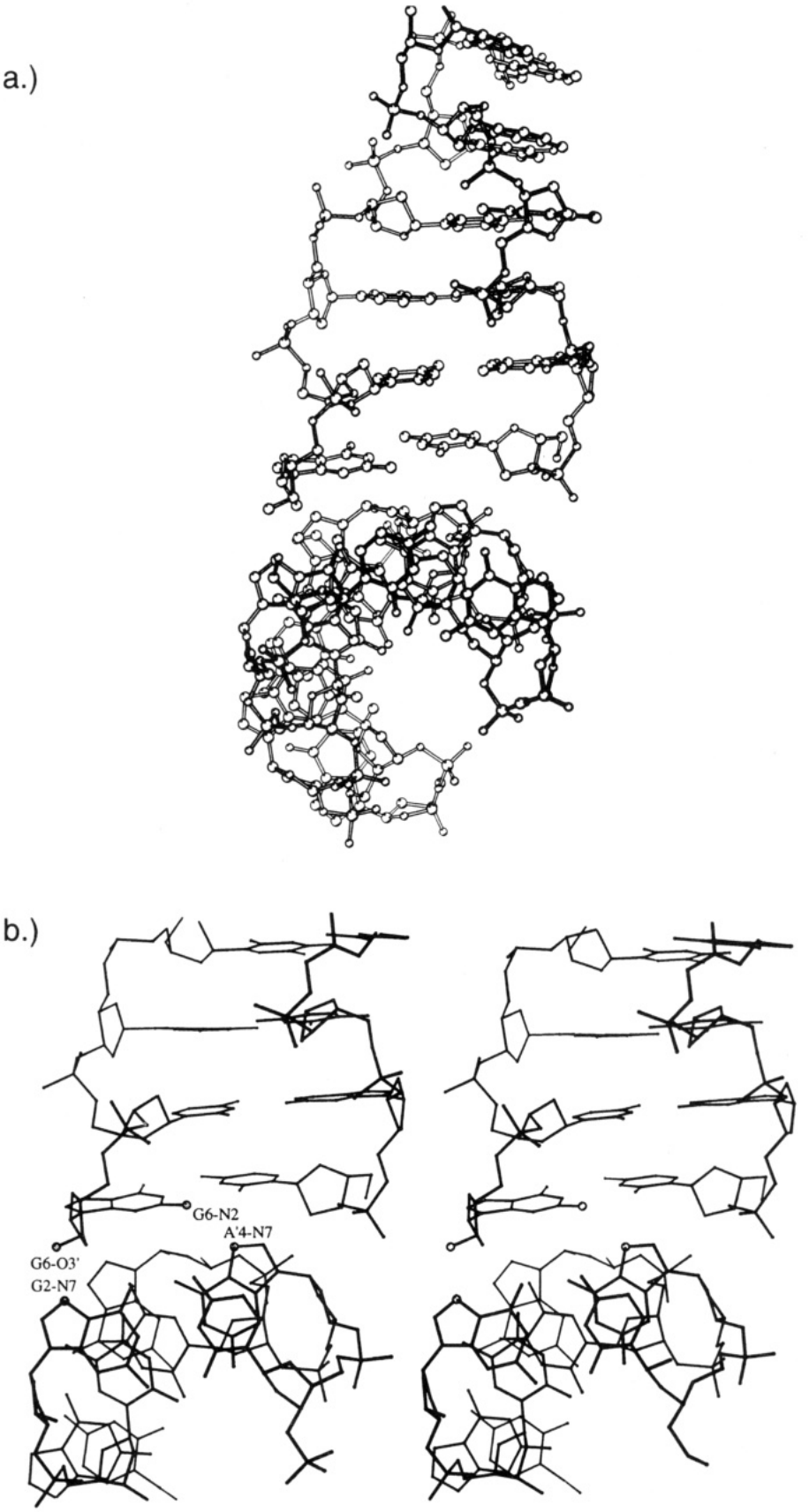

FIGURE 5: (a) Molecular packing diagram for d[CGTA′CG]$_2$ showing another view of the interactions of the helices in the crystal. (b) Stereodiagram showing the hydrogen bonding between G2-N7 and G6-O3′, and between G6-N2 and A′4-N7.

The formation of a ZII phosphate backbone conformation stabilized by intermolecular water bridges was postulated by Schneider et al. (1992) as an important prerequisite for three-dimensional crystal order in Z-DNA structures. The observation of the ZI, ZII conformational mix in the phosphate backbone at step 4−5 in d[CGTA′CG] was unexpected, as a single strand constitutes the asymmetric unit. A spermine molecule which lies along the phosphate backbone forms a

hydrogen bond (2.5 Å) to a phosphate oxygen in the ZII conformation. Spermine contacts the O1P ZII with 40% occupancy, which is the same occupancy as the refined ZII conformation. The different packing arrangement in this crystalline form precludes the possibility of single bridging waters between phosphates to presumably stabilize the ZII backbone conformation; instead, spermine fulfills this requirement. The introduction of the platinum triammine, which would make short contacts to the O1P in the ZII conformation, leads to a reduction in the population of this conformation. As a result, the observed amount of spermine is decreased.

Some aspects of the hydration pattern, such as the minor groove spine, are similar to those seen in other Z-DNA structures. However, the packing of the duplexes is very different. In other Z-DNA crystals, the duplexes are stacked end-to-end, and the phosphate backbones are linked across parallel strands by water bridges. In contrast, the crystals of d[CGTA′CG] lack both end-to-end stacking and the water bridges. Rather the flat end of one molecule is juxtaposed with the major groove of another (Figure 4a). This packing is against the slightly unwound helix in the region of A′ 4 and is accompanied by hydrogen bonding with the terminal base. The unwinding of the helix is facilitated by the roll into the major groove, which also directs the methyl group of T3 toward the purine ring of a symmetry-related molecule. It is interesting to note that these changes are in the region of the base pair substitution of TA′ for CG.

Platinum binding produced only minor changes in the DNA and water structure. These small modifications were accommodated without disturbing the local conformation. The extent of useful diffraction data, 1.60 Å resolution, was slightly reduced. Distortions associated with binding of platinum moieties were investigated by NMR methods (Marcelis et al., 1982). These studies showed that while DNA trinucleotides are structurally modified upon the binding of the Pt moieties, longer sequences of DNA undergo only minor distortions, as has been observed in the d[CGTA′CG] crystal structure.

This DNA hexamer proved to be a difficult structure to determine using molecular replacement. With similar overlapping patterns of Patterson vectors, the stacked bases and the difficulties in distinguishing between inter- and intramolecular vectors proved to be obstacles. The 12° underwinding of the DNA helix observed in the resulting structure may account for the difficulty in determining an unambiguous molecular replacement solution. The success of the analysis demonstrates the usefulness of this platinum reagent for single isomorphous replacement in DNA crystals.

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