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Molecular structure of (m^5 dC-dG)3: the role of the methyl group on 5-methyl cytosine in stabilizing Z-DNA

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ABSTRACT

The hexamer $(m^5dC-dG)_3$ has been synthesized and its three-dimensional structure determined by a single crystal X-ray diffraction analysis. The structure has been refined to a final R value of 15.6% at 1.3 Å resolution. The molecule forms a left-handed Z-DNA helix which is similar to the unmethylated Z-DNA structure. The presence of the methyl group has resulted in slight changes in the twist angle between successive base pairs and modification of some of the interatomic contacts. Methylation of cytosine in the C5 position is associated with a relative destabilization of the B-DNA structure and a stabilization through hydrophobic bonding of the Z-DNA structure.

INTRODUCTION

Z-DNA is a left-handed conformation of the DNA double helix which is favored by sequences with alternating pyrimidines and purines, especially alternating cytosine and guanine residues. The structure was first seen in a crystal X-ray diffraction analysis of a self-complementary hexanucleoside pentaphosphate $(dC-dG)_3$ at atomic resolution.[1] The conformational change leading to Z-DNA was initially observed in poly(dG-dC) in the presence of molar quantities of sodium chloride by Pohl and Jovin.[2] It has been established that the high-salt form of poly(dG-dC) is the left-handed Z-DNA.[3] DNA is a dynamically active molecule in which the right-handed B-DNA conformation is in equilibrium with left-handed Z-DNA conformation. However, the actual equilibrium is determined by a large number of parameters including the presence of various cations in the environment and covalent modifications of DNA. One of the most frequent modifications associated with gene inactivation is the methylation of cytosine residues on the C5 position when that residue precedes a guanine. The sequence m^5dC-dG is associated with systems which have decreased transcription levels.[4,5] Removal of the methyl group is associated with increased level of transcription. The relationship of this chemical modification to the B-Z equilibrium was first explored by

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Behe and Felsenfeld who synthesized and studied the molecule poly(dG-m^5dC).[6] They discovered that this molecule had a very strong tendency to form Z-DNA, much more than the unmethylated polymer. Small amounts of divalent cations such as magnesium or even traces of polyamines had the effect of shifting the equilibrium strongly toward the Z-DNA conformation. The magnitude of the change is so great it seemed likely that this stability might be expressed in some manner in the three-dimensional structure of methylated Z-DNA. To explore this, we have synthesized a hexanucleoside pentaphosphate with alternating C and G residues in which all the cytosine groups are methylated [d(m^5CpGpm^5CpGpm^5CpG) or (m^5dC-dG)$_3$]. The molecule has been crystallized and its three-dimensional structure solved. We find that the methyl group has brought about a slight modification in the structure of Z-DNA. The methyl group is found in a recessed region on the surface of the molecule in which it is in van der Waal's contact with hydrophobic elements on the molecular surface. This conformation, in contrast to the position of the methyl group in B-DNA, may explain the strong tendency for stabilizing the Z-DNA conformation.

EXPERIMENTAL

Synthesis of the methylated hexamer has been described previously.[7] The hexamer was dissolved in a solution containing 30 mM sodium cacodylate buffer (pH 7.0), 3 mM spermine.4HCl, 4 mM $MgCl_2$ and 2 mM (m^5dC-dG)$_3$. A 30 µl droplet was placed in the depression of a spot plate which was then equilibrated in a closed container with a solution containing 10% 2-methyl-2,4-pentanediol at room temperature. Crystals began to appear after a two-week period. The crystals have the form of thin plates with an elongated hexagonal cross-section. The unit cell constants and space group are listed in Table I together with the comparable data from the crystal structure of the non-methylated hexamer. The space group of the two crystals is identical and the cell constants are very similar. However, in the (m^5dC-dG)$_3$ crystal the b axis is 1 Å shorter and the c axis 1 Å longer than the non-methylated hexamer crystal. Three dimensional x-ray diffraction data were collected at -8°C on a Nicolet P3 diffractometer using a crystal with dimensions of 0.7 x 0.5 x 0.15 mm. 4208 reflections were found to be observable at the 1.5 σ(I) level. Due to the thinness of the crystal, the diffraction data was collected to a resolution of only 1.3 Å, although this was not the limit of the observed reflections. The non-methylated hexamer formed a chunkier crystal and it diffracted to a resolution of 0.9 Å. The similarity in the space group and

cell constants led us to infer that the structure was likely to be very similar to that of the non-methylated hexamer. The structure was thus solved by placing the non-methylated hexamer in the methylated hexamer lattice and this was used as a model with which to start refinement. Using a 3 Å data set, the initial R value of this structure without its surrounding water molecules was 41.6%. The Konnert-Hendrickson restraint refinement was used in the calculations.[8] After a few cycles of refinement the R factor fell rapidly and the methyl groups appeared in the electron density map together with a large number of water molecules. The final R value for the 1.3 Å observed data is 15.6%. In addition to the hexamer, the lattice contains one spermine molecule, two magnesium ions and 98 water molecules. It should be pointed out that the hexanucleoside pentaphosphate duplex itself has 10 negative charges. One spermine and two magnesium ions constitute only 8 positive charges. Hence it is likely that there exists in the lattice an additional magnesium ion or two sodium ions which we have not yet identified.

RESULTS

The molecule has a structure which is grossly similar to that found in the unmethylated hexamer. The two strands form an antiparallel double helix with Watson-Crick base pairs between the bases, the helix is left-handed and the guanine residues are in the *syn* conformation while the cytosine residues are in the *anti* conformation. Further, the guanosine residues have a C3' *endo* ring pucker while the cytidine residues have a C2' *endo* ring pucker. The quantitative comparison of these features with the comparable features in the non-methylated hexamer are presented in Table I. It can be seen that there are slight changes in the relative positions of adjacent base pairs between the two structures and also some differences in the ring pucker. The major difference is in the relative flatness of the C3' *endo* conformation of the deoxyribose ring of deoxyguanosine in the methylated polymer compared to the non-methylated polymer. This is shown by the difference in the amplitude of the pseudorotation parameter τ_m.[9] The reason for the change in the conformation in the deoxyribose ring is due to its interaction with the methyl group as described below. The helical twist angle is the angle between a line connecting C1' of one base and C1' of the paired base and the similar line of the next base pair. In regular B-DNA the helical twist angle is the same for each successive base pair. In Z-DNA, however, the helical twist angle is quite different for the sequence CpG or GpC. For the unmethylated polymer the twist angle for the CpG pair of bases is only $-8°$, compared to the rather

Table I

Comparison of $(m^5dC\text{-}dG)_3$ and $(dC\text{-}dG)_3$

	$(m^5dC\text{-}dG)_3$	$(dC\text{-}dG)_3$
Cell Constants		
a (Å)	17.76	17.88
b (Å)	30.57	31.55
c (Å)	45.42	44.58
Space Group	$P2_12_12_1$	$P2_12_12_1$
Helical Twist Angle ($\pm\sigma$)		
CpG	-13° (1)	-8° (1)
GpC	-46° (1)	-51° (2)
Sugar conformation+*		
deoxycytidine	C2'-endo δ=141° (P=149°, τ_m= 41°)	C2'-endo δ=146° (P=153°, τ_m= 35°)
Deoxyguanosine (excluding terminal)	C3'-endo δ= 94° (P= 30°, τ_m= 19°)	C3'-endo δ= 97° (P= 27°, τ_m= 31°)
Glycosyl orientation+		
cytosine	Anti χ =-157°	Anti χ =-151°
guanine	Syn χ = 69°	Syn χ = 67°

α β γ δ ε ζ

+ : Torsional angles are defined as 03'--P--05'--C5'--C4'--C3'--03'--P--05' and χ is the glycosyl torsion angle.

* : P and τ_m are, respectively, the phase angle of pseudorotation and the degree of pucker.[9]

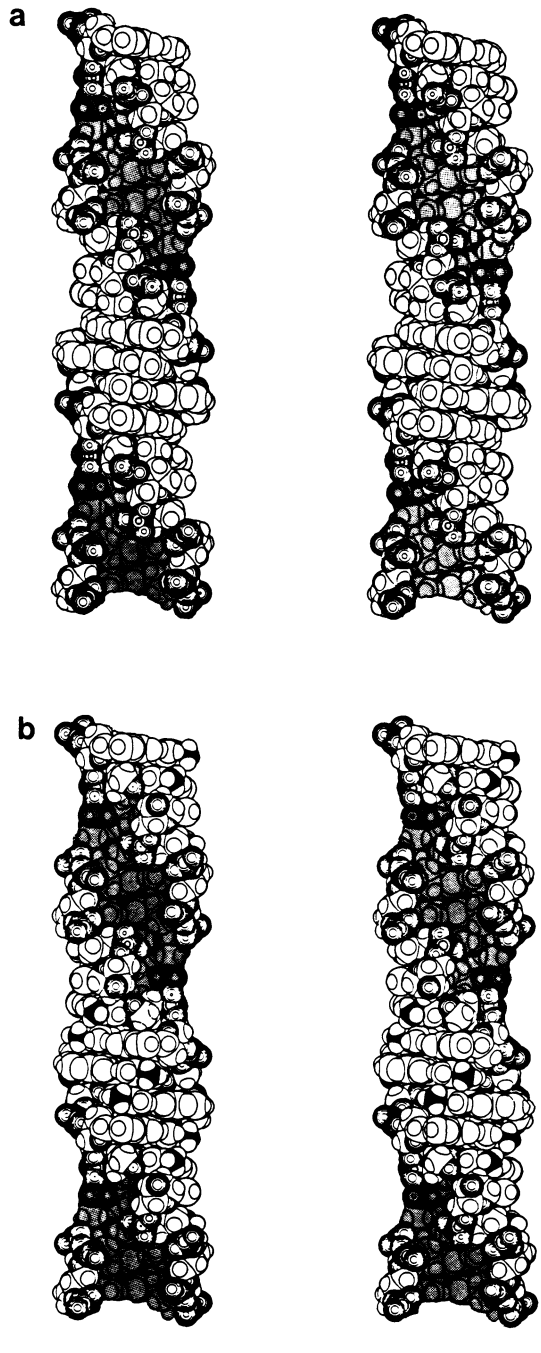
large rotation seen in the GpC base pair (-51°). There has been a slight change in this twist angle for the methylated hexamer in that the CpG twist angle is increased to -13° while the GpC angle has correspondingly changed to -46°. The net effect of these changes in the twist angle is to bring the two methyl groups closer together in the methylated polymer.

The overall effect of these changes can be seen in Fig. 1, which contains stereo diagrams of three successive hexamer elements as they appear in the crystal lattice. The diagrams show the non-methylated and methylated hexamer crystals. The double helix is interrupted in the sugar-phosphate chain by the absence of every sixth phosphate. However, as in the original non-methylated hexamer structure, the paired nucleosides form an uninterrupted

double helix along the c axis. The original hexamer has a form which looks very similar to that found in the elongated fiber of Z-DNA,[10,11] and a similar situation is found for the methylated polymer. In Fig. 1b it can be seen that the methyl groups on the opposite strands are rather close to each other. The carbon atoms are 4.6 Å apart, almost in van der Waal's contact. If one placed a methyl group on the C5 position of cytosine in the unmethylated structure, the two methyl groups would be a distance of 5.2 Å apart. Thus a shortening of almost 0.6 Å in the distance between methyl groups on opposite strands is associated with the change in the twist angle described above.

The stereo diagram shows that the methyl group occupies a somewhat protected position, recessed slightly on the surface of the molecule so that it is under the imidazole group of guanine with which it is in van der Waal's contact (3.4 Å). The methyl group is 3.6-3.8 Å away from carbon C2' of guanosine and 4.2 Å from C1'. There is thus a close contact between the methyl group and the sugar residue of the adjacent guanosine(5'-side)as well as its imidazole ring. This close contact can be seen in Fig. 1b. The reason for the slight conformational change of the methylated hexamer compared to the non-methylated molecule can be seen if one were to place a methyl group on the 5 position of cytosine on the unmethylated polymer. When such a structure is made, the distance between this methyl group and guanosine C2' is 3.2 Å, which is too short a distance for a van der Waal's contact. That distance is relaxed in the actual structure of the methylated polymer to a distance of 3.6 to 3.8 Å in the various residues. In order to relieve an unacceptable van der Waal's contact, the molecule has readjusted itself slightly and produced a change in the helix twist angle and flattened the guanosine sugar ring somewhat.

The disposition of the methyl group of 5 methylcytosine in Z-DNA compared to the position which that methyl group occupies in right-handed B-DNA is illustrated in the stacking diagrams shown in Fig. 2. These diagrams show end views of the sequences CpG and GpC both in the methylated Z-DNA polymer as well as in B-DNA. The van der Waal's diagrams are viewed down the helix axis. The upper base pair has different atoms indicated by shading, but the lower base pair shows only the outline of the bases. The methyl carbon atom of the upper base pair is solid black while the methyl carbon atom on the lower base pair is shaded gray. If one looks at the sequence CpG of Z-DNA, the methyl groups on the two base pairs are fairly close together. Further, there is a limited accessibility to the methyl group by solvent water molecules which would be found at the top of the base pair in the diagram. The accessibility


a
b

to the gray methyl group of the bottom base pair is limited because of the presence of the amino group and carbonyl oxygen atom of the upper base pair. This situation is in marked contrast to the methyl group of the CpG sequence in B-DNA which is shown in lower part of Figure 2. It can be seen that the methyl group B-DNA is thrust out strongly into the solvent region so that water molecules have access not only to it but to contiguous sections of the cytosine ring. A difference in accessibility of the GpC residues is shown in the other two diagrams. In the Z conformation the methyl group of the upper base pair is in van der Waal's contact with the imidazole group of the guanine below it which projects considerably further away from the center of the molecule than the methyl group. In the GpC sequence of B-DNA, the methyl group is somewhat protected by the imidazole group of guanine below it but the protection is less than that seen in the Z-DNA structure. The methyl group in the Z-DNA structure is thus recessed somewhat into a slight depression on the surface of the molecule as shown in Fig. 1b and 2, and this is in marked contrast to the position of that methyl group in B-DNA where it is thrust out into the solvent and thus it has considerably greater accessibility to water molecules. This strongly suggests that the Z-DNA methyl group making close van der Waal's contact with both the imidazole group of guanine and the carbon atoms of the sugar residue of guanosine is stabilized by hydrophobic interactions to these residues and is somewhat shielded from surrounding water molecules. In contrast, the methyl group on C5 of cytosine in B-DNA has a much greater surface area accessible to solvent water molecules.

Another aspect of the difference in hydration between methylated and non-methylated Z-DNA is illustrated in Fig. 3, which shows the electron

Figure 1: Stereo diagrams of a portion of the Z-DNA helix found both in the methylated and unmethylated hexamer crystals. Van der Waal's models are drawn in which the oxygens in the backbone are indicated by circles and the phosphate groups by circles with crossed lines.

1A: A stereo view of the non-methylated polymer.[11] Note that there is a slight depression seen on the convex outer surface of the molecule due to the fact that the guanine imidazole rings project further away from the axis than the cytosine rings.

1B: Stereo diagram of the methylated hexamer. The methyl groups are shaded solid black. Three hexamer segments are shown in each diagram just as they appear in the crystal. Every sixth phosphate group is missing because the molecule in the crystal is a hexanucleoside pentaphosphate. Note that the methyl groups are close together and that they fill part of the depression on the surface caused by the overhanging imidazole rings of guanine which protrudes from the center of the molecule. This can be seen easily at the side of the molecule where the methyl groups effectively fill a depression which is visible in the non-methylated polymer of Figure 1A.

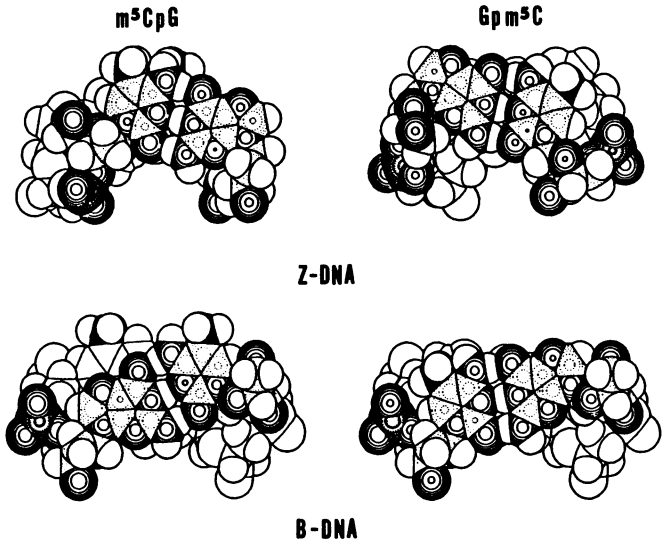


Figure 2: Fragments of Z-DNA and B-DNA are shown containing two base pairs with the sequences m^5CpG and Gpm^5C. The base pair closer to the reader has shaded atoms, while the base pair away from the reader has the atoms shown only in outline. The methyl group on 5-methylcytosine in the upper base pair closer to the reader is solid black, while the methyl group attached to the lower base pair is shaded gray. The two diagrams at the bottom show the methyl groups in B-DNA which are more exposed to solvent water molecules than are the methyl groups in the upper two base pairs in Z-DNA.

density map found in a 3 Å thick section through a GC base pair in the Z-DNA helix. A water molecule W1 is seen which is hydrogen bonding to the amino group in the N4 position of cytosine. This water molecule is at a distance of 2.9 Å away from the amino group. The water molecule forms an angle of 145° between W1---N4 and the C4-N4 bond of the cytosine residue. The amino group is in the planar trigonal conformation so one would anticipate that this would adopt an angle close to 120°. In the non-methylated structure that same water molecule is found hydrogen bonded to the N4 position of cytosine, but in that structure the angle between the C4-N4 bond of the cytosine and the water---N4 hydrogen bond is 112°. Thus the presence of the methyl group has effectively moved the water molecule away from the position occupied by the methyl group. This is further evidence that the packing of water molecules is different in the primary hydration shell of the methylated form of Z-DNA and the non-methylated form.

The atomic coordinates of the molecule are listed in Table II. Two of

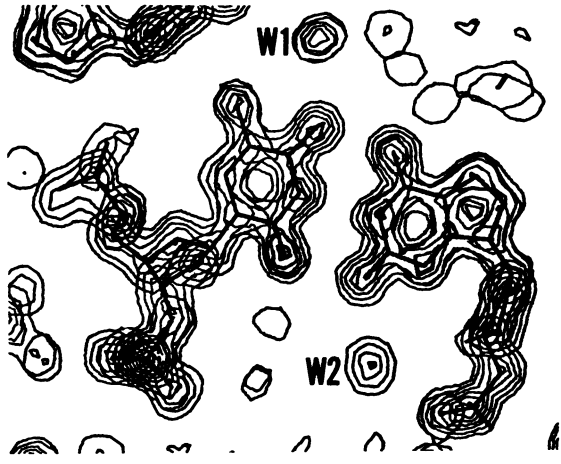


Figure 3: An electron density map is shown for a section of the methylated polymer which encloses one base pair. The electron density map covers a 3 Å thick section of the map perpendicular to the c axis. The 5-methylcytosine of the base pair is shown on the left and the guanosine residue on the right. A water molecule W1 is hydrogen bonded to the amino group on the 4 position of cytosine. The presence of the methyl group nearby forces that water molecule to occupy a position closer to the line of the cytosine C4 N4 bond than is the case in the stucture of the non-methylated polymer. The second water molecule W2 receives a hydrogen bond from N2 of guanine and donates a hydrogen bond to the phosphate group on the 3' position of the guanosine. W2 has been described previously[1] and stabilizes the syn conformation of guanine in Z-DNA.

the 10 phosphate groups attached to residues m^5C3 and m^5C5 are found in two different conformations, which have previously been identified as Z_I and Z_{II}.[11] They occur in roughly equal populations in this crystal form. Z-DNA is known to exist as a family of closely related structures.[11,12]

DISCUSSION

The principal result of this structure determination is the demonstration that the methylated form of poly(dG-dC) has a three-dimensional structure which is very similar to the unmethylated form. Both of them form Z-DNA molecules even though there are some minor rearrangements which are needed to accommodate the additional methyl group. This overall similarity is in agreement with the fact that antibodies raised against Z-DNA produced by a non-methylated polymer have the ability to react with both the methylated polymer as well as the non-methylated polymer.[13] Thus the small differences in conformation between the methylated and non-methylated polymer are not detected by polyclonal antibodies against Z-DNA. On the other hand it has

Table II
Atomic Coordinates in Fractions of the Unit Cell Edge

		X	Y	Z			X	Y	Z
C	1C1'	0.5910	0.4672	-0.0683	C	3C1'	0.6213	0.5707	0.0981
C	1C2'	0.5579	0.4809	-0.0991	C	3C2'	0.6273	0.5908	0.0655
C	1C3'	0.4894	0.5072	-0.0885	C	3C3'	0.6392	0.6387	0.0750
C	1C4'	0.4660	0.4852	-0.0591	C	3C4'	0.5859	0.6451	0.1018
C	1C5'	0.3988	0.4541	-0.0605	C	3C5'	0.5104	0.6654	0.0958
C	103'	0.5173	0.5488	-0.0820	C	303'	0.7128	0.6467	0.0840
C	101'	0.5283	0.4595	-0.0498	C	301'	0.5719	0.5997	0.1118
C	105'	0.4154	0.4232	-0.0842	C	305'	0.4807	0.6438	0.0720
C	1N1	0.6363	0.4251	-0.0715	C	3N1	0.5846	0.5270	0.0966
C	1C2	0.7112	0.4304	-0.0765	C	3C2	0.6306	0.4918	0.0924
C	1N3	0.7520	0.3921	-0.0792	C	3N3	0.5966	0.4521	0.0902
C	1C4	0.7209	0.3507	-0.0767	C	3C4	0.5196	0.4464	0.0917
C	1C5	0.6433	0.3476	-0.0710	C	3C5	0.4732	0.4839	0.0957
C	1C6	0.6035	0.3842	-0.0686	C	3C6	0.5073	0.5225	0.0976
C	102	0.7432	0.4650	-0.0793	C	302	0.6979	0.4946	0.0907
C	1N4	0.7687	0.3157	-0.0802	C	3N4	0.4914	0.4049	0.0893
C	1M5	0.6065	0.3029	-0.0680	C	3M5	0.3885	0.4807	0.0982
G	2P	0.4723	0.5928	-0.0865	G	4P	0.7492	0.6946	0.0799
G	201P	0.4256	0.5853	-0.1125	G	402P	0.8227	0.6836	0.0873
G	202P	0.5252	0.6255	-0.0815	G	401P	0.7167	0.7152	0.0546
G	2C1'	0.3231	0.5301	0.0146	G	4C1'	0.5723	0.7304	0.1793
G	2C2'	0.3472	0.5637	0.0381	G	4C2'	0.6285	0.7333	0.2053
G	2C3'	0.3994	0.5942	0.0227	G	4C3'	0.7035	0.7274	0.1909
G	2C4'	0.3757	0.5911	-0.0101	G	4C4'	0.6923	0.7375	0.1575
G	2C5'	0.4451	0.5974	-0.0301	G	4C5'	0.7395	0.7085	0.1370
G	203'	0.3937	0.6402	0.0283	G	403'	0.7617	0.7552	0.1995
G	201'	0.3440	0.5491	-0.0135	G	401'	0.6156	0.7312	0.1519
G	205'	0.4147	0.5977	-0.0585	G	405'	0.7198	0.7220	0.1090
G	2N1	0.5673	0.4393	0.0149	G	4N1	0.5627	0.5619	0.1762
G	2C2	0.5579	0.4830	0.0138	G	4C2	0.6226	0.5886	0.1765
G	2N3	0.4933	0.5053	0.0141	G	4N3	0.6230	0.6329	0.1780
G	2C4	0.4336	0.4784	0.0154	G	4C4	0.5515	0.6485	0.1783
G	2C5	0.4339	0.4338	0.0170	G	4C5	0.4858	0.6251	0.1773
G	2C6	0.5061	0.4134	0.0162	G	4C6	0.4929	0.5786	0.1762
G	2N7	0.3634	0.4155	0.0183	G	4N7	0.4235	0.6513	0.1770
G	2C8	0.3203	0.4511	0.0168	G	4C8	0.4537	0.6909	0.1785
G	2N9	0.3577	0.4890	0.0159	G	4N9	0.5278	0.6920	0.1791
G	2N2	0.6147	0.5064	0.0123	G	4N2	0.6876	0.5730	0.1768
G	206	0.5147	0.3720	0.0166	G	406	0.4391	0.5526	0.1751
C	3P	0.4627	0.6675	0.0401	C	5P	0.7860	0.7595	0.2327
C	302P	0.4377	0.7092	0.0481	C	501P	0.7192	0.7695	0.2508
C	301P	0.5272	0.6650	0.0205	C	502P	0.8504	0.7856	0.2324
C	3P *	0.4013	0.6595	0.0592	C	5P *	0.8400	0.7367	0.2107
C	301P*	0.3838	0.7067	0.0570	C	501P*	0.8761	0.7099	0.1881
C	302P*	0.3527	0.6322	0.0779	C	502P*	0.8791	0.7746	0.2220

Table II (continued)

		X	Y	Z			X	Y	Z
C	5C1'	0.7721	0.6037	0.2663	C	7O1'	0.5255	0.4342	0.3089
C	5C2'	0.8136	0.6074	0.2354	C	7O5'	0.4004	0.4109	0.3437
C	5C3'	0.8869	0.6267	0.2461	C	7N1	0.5653	0.5021	0.3281
C	5C4'	0.8663	0.6567	0.2726	C	7C2	0.6262	0.5279	0.3320
C	5C5'	0.8619	0.7057	0.2656	C	7N3	0.6108	0.5722	0.3343
C	5O3'	0.9325	0.5939	0.2581	C	7C4	0.5410	0.5908	0.3330
C	5O1'	0.7945	0.6421	0.2806	C	7C5	0.4795	0.5623	0.3281
C	5O5'	0.8168	0.7105	0.2406	C	7C6	0.4942	0.5192	0.3258
C	5N1	0.6877	0.6018	0.2627	C	7O2	0.6914	0.5149	0.3340
C	5C2	0.6604	0.5602	0.2582	C	7N4	0.5366	0.6349	0.3359
C	5N3	0.5848	0.5559	0.2540	C	7M5	0.4004	0.5798	0.3262
C	5C4	0.5346	0.5907	0.2549	G	8P	0.6464	0.3141	0.3436
C	5C5	0.5643	0.6333	0.2603	G	8O1P	0.7279	0.3097	0.3417
C	5C6	0.6393	0.6375	0.2638	G	8O2P	0.6041	0.3000	0.3679
C	5O2	0.7015	0.5282	0.2574	G	8C1'	0.4747	0.3030	0.2409
C	5N4	0.4604	0.5819	0.2512	G	8C2'	0.5379	0.2960	0.2179
C	5M5	0.5124	0.6723	0.2616	G	8C3'	0.6072	0.2876	0.2347
G	6P	1.0210	0.5932	0.2549	G	8C4'	0.5817	0.2799	0.2673
G	6O1P	1.0371	0.6082	0.2254	G	8C5'	0.6352	0.3019	0.2890
G	6O2P	1.0390	0.5511	0.2663	G	8O3'	0.6476	0.2499	0.2254
G	6C1'	1.0010	0.7034	0.3494	G	8O1'	0.5069	0.2950	0.2696
G	6C2'	1.0313	0.6674	0.3702	G	8O5'	0.6000	0.2914	0.3166
G	6C3'	1.1026	0.6533	0.3569	G	8N1	0.5310	0.4668	0.2448
G	6C4'	1.0876	0.6593	0.3228	G	8C2	0.5788	0.4330	0.2441
G	6C5'	1.0628	0.6177	0.3079	G	8N3	0.5607	0.3905	0.2429
G	6O3'	1.1653	0.6802	0.3646	G	8C4	0.4854	0.3855	0.2426
G	6O1'	1.0280	0.6913	0.3203	G	8C5	0.4306	0.4168	0.2428
G	6O5'	1.0584	0.6281	0.2785	G	8C6	0.4567	0.4610	0.2447
G	6N1	0.7443	0.6252	0.3447	G	8N7	0.3594	0.3996	0.2423
G	6C2	0.8143	0.6090	0.3451	G	8C8	0.3724	0.3567	0.2415
G	6N3	0.8802	0.6301	0.3464	G	8N9	0.4453	0.3462	0.2412
G	6C4	0.8680	0.6740	0.3473	G	8N2	0.6480	0.4399	0.2455
G	6C5	0.8009	0.6954	0.3467	G	8O6	0.4133	0.4940	0.2459
G	6C6	0.7343	0.6690	0.3451	C	9P	0.7327	0.2540	0.2159
G	6N7	0.8091	0.7395	0.3480	C	9O1P	0.7765	0.2781	0.2373
G	6C8	0.8826	0.7448	0.3492	C	9O2P	0.7512	0.2116	0.2058
G	6N9	0.9216	0.7071	0.3492	C	9C1'	0.7435	0.3933	0.1598
G	6N2	0.8233	0.5683	0.3440	C	9C2'	0.7751	0.3842	0.1916
G	6O6	0.6677	0.6834	0.3446	C	9C3'	0.8420	0.3545	0.1830
C	7C1'	0.5827	0.4542	0.3257	C	9C4'	0.8120	0.3280	0.1568
C	7C2'	0.5743	0.4322	0.3569	C	9C5'	0.7855	0.2815	0.1642
C	7C3'	0.5623	0.3841	0.3465	C	9O3'	0.9030	0.3792	0.1726
C	7C4'	0.5135	0.3885	0.3188	C	9O1'	0.7485	0.3522	0.1454
C	7C5'	0.4301	0.3810	0.3225	C	9O5'	0.7288	0.2819	0.1846
C	7O3'	0.6321	0.3655	0.3412	C	9N1	0.6641	0.4083	0.1622

Table II (continued)

	X	Y	Z		X	Y	Z
C 9C2	0.6555	0.4526	0.1670	C 11C4'	1.0311	0.4382	-0.0169
C 9N3	0.5832	0.4677	0.1690	C 11C5'	1.0915	0.4031	-0.0122
C 9C4	0.5195	0.4419	0.1672	C 1103'	1.0100	0.5100	-0.0022
C 9C5	0.5315	0.3962	0.1623	C 1101'	0.9602	0.4185	-0.0255
C 9C6	0.6019	0.3816	0.1598	C 1105'	1.0658	0.3751	0.0096
C 902	0.7093	0.4772	0.1690	C 11N1	0.8383	0.4063	-0.0068
C 9N4	0.4510	0.4609	0.1701	C 11C2	0.7690	0.4245	-0.0022
C 9M5	0.4662	0.3646	0.1595	C 11N3	0.7106	0.3962	0.0012
G 10P	0.9874	0.3636	0.1743	C 11C4	0.7165	0.3507	0.0012
G 1002P	0.9999	0.3396	0.2000	C 11C5	0.7892	0.3335	-0.0030
G 1001P	1.0324	0.4043	0.1688	C 11C6	0.8465	0.3613	-0.0072
G 10C1'	0.9134	0.2695	0.0715	C 1102	0.7580	0.4637	-0.0017
G 10C2'	0.9549	0.2967	0.0483	C 11N4	0.6525	0.3274	0.0058
G 10C3'	1.0033	0.3276	0.0645	C 11M5	0.8013	0.2848	-0.0037
G 10C4'	1.0073	0.3116	0.0977	G 12P	1.0740	0.5464	-0.0009
G 10C5'	1.0009	0.3491	0.1192	G 1201P	1.0385	0.5870	-0.0117
G 1003'	1.0789	0.3288	0.0547	G 1202P	1.1015	0.5403	0.0279
G 1001'	0.9475	0.2806	0.1002	G 12C1'	1.1889	0.4500	-0.0935
G 1005'	0.9964	0.3287	0.1477	G 12C2'	1.1650	0.4878	-0.1145
G 10N1	0.6976	0.3801	0.0823	G 12C3'	1.2056	0.5258	-0.1014
G 10C2	0.7712	0.3871	0.0791	G 12C4'	1.1997	0.5162	-0.0676
G 10N3	0.8267	0.3567	0.0759	G 12C5'	1.1343	0.5387	-0.0517
G 10C4	0.7968	0.3161	0.0767	G 1203'	1.2840	0.5286	-0.1100
G 10C5	0.7239	0.3041	0.0805	G 1201'	1.1933	0.4695	-0.0645
G 10C6	0.6703	0.3392	0.0834	G 1205'	1.1472	0.5312	-0.0204
G 10N7	0.7140	0.2601	0.0812	G 12N1	0.9106	0.4049	-0.0898
G 10C8	0.7833	0.2456	0.0773	G 12C2	0.9426	0.4451	-0.0906
G 10N9	0.8356	0.2764	0.0747	G 12N3	1.0160	0.4556	-0.0920
G 10N2	0.7969	0.4247	0.0787	G 12C4	1.0585	0.4188	-0.0915
G 1006	0.6007	0.3347	0.0871	G 12C5	1.0351	0.3764	-0.0902
C 11P	1.1129	0.3727	0.0411	G 12C6	0.9552	0.3693	-0.0896
C 1101P	1.1037	0.4083	0.0619	G 12N7	1.0931	0.3472	-0.0898
C 1102P	1.1822	0.3568	0.0285	G 12C8	1.1535	0.3732	-0.0915
C 11C1'	0.9004	0.4391	-0.0103	G 12N9	1.1369	0.4155	-0.0925
C 11C2'	0.9367	0.4506	0.0206	G 12N2	0.9012	0.4778	-0.0906
C 11C3'	1.0130	0.4677	0.0104	G 1206	0.9255	0.3316	-0.0883

been shown that some monoclonal antibodies raised against a non-methylated Z-DNA polymer will not react with the methylated Z-DNA polymer, presumably because of the interference associated with the recognition of the non-methylated polymer on the outer surface of the molecule where the methyl group is located.[14]

As seen in the stereo diagrams of Fig. 1, the methyl groups on the methylated Z-DNA hexamer occupy a position on the surface of the molecule where they are relatively close together and somewhat recessed, tucked under

the imidazole groups and sugar-carbon atoms of the guanosine sugars. Fig. 2 shows that these methyl groups are relatively less accessible to surrounding solvent molecules than are the corresponding methyl groups in the B-DNA conformation. In Z-DNA, the methyl groups are in close van der Waal's contacts with both the imidazole ring of guanine and the carbon atoms of the sugar and they constitute in essence a small stabilizing hydrophobic patch on the surface of the molecule. This is in contrast to the position of the methyl group in B-DNA on cytosine C5 which protrudes into the aqueous solvent phase from the major groove of the B-DNA helix. We suggest that the stabilization of Z-DNA on methylation[6] relative to B-DNA derives from two distinct components. One is a destabilization of B-DNA due to the methyl group in the major groove interacting with water molecules and, secondly, a stabilization of Z-DNA itself through the formation of a hydrophobic patch on the surface of the molecule in which the methyl group fills a slight depression in the surface of the Z-DNA helix.

In vivo, especially for higher eukaryotes, the effects of methylation of CpG sequences in DNA is associated with an inhibition of RNA synthesis.[4,5] Behe and Felsenfeld have shown that Z-DNA formation in poly(dG-dm^{5}C) is facilitated by small amounts of cations, especially the polyamines.[6] In their experiments, it took three orders of magnitude fewer magnesium ions to convert B-DNA to Z-DNA in the methylated polymer. Similarly, spermine stabilized the formation of Z-DNA for the methylated polymer in submicromolar concentrations. These results support the concept that methylation of alternating dC-dG sequences may induce the formation of Z-DNA, perhaps even in short segments of DNA. The present structural studies provide a rationale for understanding the mechanism for this stabilization. What is not answered in the present study is the question regarding how small a segment of Z-DNA can be formed given the stimulus that methylation of cytosine residues has for Z-DNA formation. We would like to be able to answer the question as to whether the methylation of CpG sequences which occurs *in vivo* actually results in the formation of small stretches of Z-DNA. The present structural study shows us that the destabilization of B-DNA and the relative stabilization of Z-DNA is associated with interactions which are in the immediate vicinity of the methyl groups themselves. Thus, it is conceivable that small sections of Z-DNA could form in the middle of B-DNA. However, in order to study that question it will be necessary to carry out a different kind of experiment in which small segments of a DNA oligomer are methylated in the hope that it would be possible to trap in a single crystal lattice a segment containing a B-Z-B interface. The

present study at least allows us to anticipate the structure of the methylated Z-DNA segment of such an overall conformation.

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