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Synthesis, structure and thermodynamic properties of 8-methylguanine-containing oligonucleotides: Z-DNA under physiological salt conditions

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ABSTRACT

Various oligonucleotides containing 8-methylguanine (m^8G) have been synthesized and their structures and thermodynamic properties investigated. Introduction of m^8G into DNA sequences markedly stabilizes the Z conformation under low salt conditions. The hexamer $d(CGC[m^8G]CG)_2$ exhibits a CD spectrum characteristic of the Z conformation under physiological salt conditions. The NOE-restrained refinement unequivocally demonstrated that $d(CGC[m^8G]CG)_2$ adopts a Z structure with all guanines in the *syn* conformation. The refined NMR structure is very similar to the Z form crystal structure of $d(CGCGCG)_2$, with a root mean square deviation of 0.6 between the two structures. The contribution of m^8G to the stabilization of Z-DNA has been estimated from the mid-point NaCl concentrations for the B–Z transition of various m^8G-containing oligomers. The presence of m^8G in $d(CGC[m^8G]CG)_2$ stabilizes the Z conformation by at least $\Delta G = -0.8$ kcal/mol relative to the unmodified hexamer. The Z conformation was further stabilized by increasing the number of m^8Gs incorporated and destabilized by incorporating *syn*-A or *syn*-T, found respectively in the (A,T)-containing alternating and non-alternating pyrimidine–purine sequences. The results suggest that the chemically less reactive m^8G base is a useful agent for studying molecular interactions of Z-DNA or other DNA structures that incorporate *syn*-G conformation.

INTRODUCTION

It has been well established that DNA structure has a remarkable conformational heterogeneity (1,2). Not only does the biologically relevant B-DNA exhibit considerable local heterogeneity, dramatically different DNA structures such as Z-DNA have also been discovered. While the precise biological functions of Z-DNA have yet to be identified, its role in regulating DNA supercoiling has been amply demonstrated (3,4). A recent study by Rich and colleagues has shown that chicken double-stranded RNA adenosine deaminase has strong Z-DNA binding properties (5). This enzyme is known to work near the transcription apparatus, where a high negative supercoiling density along the DNA chain exists in front of the site of polymerase action (2). Thus far most of the thermodynamic properties of Z-DNA have been obtained through the use of supercoiled DNA plasmids containing various alternating $(C{\cdot}G)_n$ inserts or their variants (2–4). However, other aspects of Z-DNA have not been thoroughly investigated, presumably due to the difficulty of obtaining stable Z form oligonucleotides in a physiological salt solution. Much of the available experimental data are limited to $d(C{\cdot}G)_n$ oligomers under non-physiological conditions of high alcohol or high salt concentrations (6–8). While some chemical modifications, such as C^5-methylation or C^5-bromination of cytosine (9) or C^8-bromination of guanine (10,11), have been shown to stabilize the Z conformation in linear DNA oligomers, they have either limited power for inducing the B–Z transition or they are chemically unstable. Therefore, it is desirable to have a more convenient and reliable way to stabilize Z form oligomers under low salt conditions by incorporating chemically and photochemically inert modified bases. We report herein that the introduction of a methyl group at the guanine C^8 position produces a stable m^8-modified guanine base and markedly stabilizes the Z conformation of short oligonucleotides of a variety of sequences under physiological salt conditions.

MATERIALS AND METHODS

Pyridine and acetonitrile (HPLC grade) were dried over calcium hydride. 2′-Deoxyguanosine (Yamasa Co.), nucleoside β-cyanoethylphosphoramidite reagents (Applied Biosystems), calf intestine alkaline phosphatase (AP) (1000 U/ml) and snake venom phosphodiesterase (s.v. PDE) (3 U/ml; Boehringer Mannheim) were all of the highest grade. Silica gel columns and thin layer chromatography were carried out on Wakogel C-200 and Merck silica gel 60 PF_{254} plates respectively. FAB mass spectra were obtained in a JEOL-JMS-SX102A.

Synthesis of m^8G-containing oligonucleotides

Introduction of a methyl group at the C^8 position of guanine was performed by the free radical methylation method (12). To a solution of *N*-isobutyryl-2′-deoxyguanosine (1.0 g, 2.97 mmol) and $FeSO_4{\cdot}7H_2O$ (6.7 g, 24.1 mmol) in 160 ml 1 N H_2SO_4 was added

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an aqueous solution (100 ml) containing 2.6 ml 70% *t*-butyl hydroperoxide (19.0 mmol) dropwise over a period of 5 min. After stirring at 0°C for 60 min the reaction mixture was neutralized with saturated KOH solution. The supernatant, obtained by centrifugation of the brown slushy mixture, was concentrated to dryness and the resulting brownish solid was triturated three times with 200 ml methanol. The combined methanol solution was concentrated and the residue was subjected to silica gel column chromatography. Elution with CH_2Cl_2/methanol (9:1) afforded 8-methyl-*N*-isobutyryl-2′-deoxyguanosine (**1**) as a white powder: yield 527 mg (51%), analytical data, m.p. 195°C (dec.); ^{1}H NMR (D_2O, 200 MHz) δ 1.21 [d, 6 H, J = 6.9 Hz, -CH(C**H**$_3$)$_2$], 2.25 (ddd, 1 H, J = 13.7, 6.9, 3.6 Hz, 2′), 2.56 (s, 3 H, -8CH_3), 2.72 [sep, 1 H, J = 6.9 Hz, -C**H**(CH_3)$_2$], 3.17 (ddd, 1 H, J = 13.7, 7.4, 6.9 Hz, 2′), 3.72 (dd, 1 H, J = 11.9, 5.4 Hz, 5′), 3.74 (dd, 1 H, J = 11.9, 4.1 Hz, 5′), 3.92 (ddd, 1 H, 5.4, 4.1, 3.6 Hz, 4′), 4.59 (ddd, 1 H, J = 6.9, 3.6, 3.6 Hz, 3′), 6.32 (dd, 1 H, J = 7.4, 6.9 Hz, 1′); FABMS (positive ion) *m/z* 352 $(M+H)^+$.

1 was dimethoxytritylated according to a standard procedure to DMTr-**1**, which was further converted to its β-cyanoethylphosphoramidite with the following analytical data: ^{1}H NMR (CD_3OD, 200 MHz) δ 1.17 [d, 12 H, J = 6.5 Hz, -NCH(C**H**$_3$)$_2$], 1.26 [dd, 6 H, J = 6.8, 3.4 Hz, -CH(C**H**$_3$)$_2$], 2.41–2.64 [m, 3 H, 2′, -NC**H**(CH_3)$_2$], 2.57 (s, 3 H, -8CH_3), 2.63–2.77 [m, 1 H, -C**H**(CH_3)$_2$], 2.69 (t, 1 H, J = 5.9, -OCH_2-), 2.84 (t, 1 H, J = 5.9, -OCH_2-), 3.28–3.54 (m, 3 H, 2′, -CH_2CN), 3.72 (s, 3 H, -OCH_3), 3.73 (s, 3 H, -OCH_3), 3.54–3.71 (m, 2 H, 5′), 4.07–4.34 (m, 1 H, 4′), 4.09 (ddd, 1 H, J = 7.2, 3.4, 3.2 Hz, 4′), 4.64–4.83 (m, 1 H, 3′), 6.36 (t, 1 H, J = 7.3 Hz, 1′), 6.60–6.80 (m, 4 H, aromatic), 7.06–7.44 (m, 9 H, aromatic); ^{31}P NMR (CD_3OD, 80 MHz) δ 148.48; FABMS (positive ion) *m/z* 854 $(M+H)^+$. A set of m^{8}G-containing DNA oligomers (**2–7**) (see Table 3) were prepared by means of an automated DNA synthesizer. After deprotection with ammonium hydroxide oligonucleotides were purified by HPLC and the composition of the nucleosides was confirmed by enzymatic digestion.

NMR analysis

The NMR solution (1 mM duplex with 0.04 M phosphate buffer, thus 0.06 M Na^+, pH 7.0, in D_2O) of d(CGC[m^{8}G]CG)$_2$ was prepared using the established procedure (13). NMR spectra were collected on a Varian VXR500 500 MHz spectrometer and processed with FELIX v1.1 on Silicon Graphics IRIS workstations. The temperature was controlled to be accurate within 0.01°C. T_1 relaxation experiments were carried out with the standard 180°–t–90° inversion–recovery sequence and the average T_1 relaxation time was 2.7 s. The non-exchangeable proton 2D NOESY spectra were collected at 2°C with a mixing time of 100 ms and a total recycle delay of 7.0 s. The data were collected by the States/TPPI technique (14) with 512 t_1 increments and 2048 t_2 complex points, each the average of 16 transients. Apodization of the data in the t_1 and t_2 dimensions consisted of 8 Hz exponential multiplication with half of a sine-squared function for the last fourth of the data to reduce truncation artifacts. Integrals from the non-exchangeable 2D NOE dataset were extracted by evaluation with the observed cross-peak shapes of each spin in the f_1 and f_2 dimensions. These shapes were determined by spectral analysis using the program MYLOR (13). The exchangeable proton 2D NOESY experiment was carried out in 90% H_2O/10% D_2O solution using the 1–1 pulse sequence (14) as the read sequence, with a mixing time of 100 ms and a recycle delay of 2.7 s, each data point the average

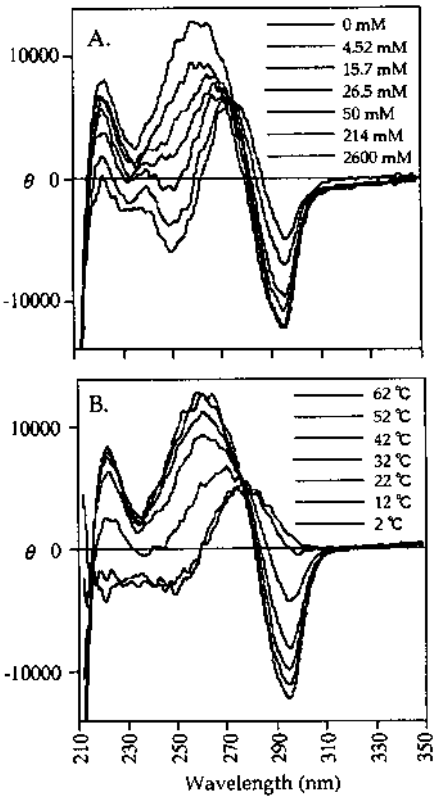

Figure 1. (**A**) CD spectra of d(CGC[^{8}mG]CG) (**5**, 0.15 mM base concentration) in 5 mM Na cacodylate buffer, pH 7.0, at 10°C at various NaCl concentrations. (**B**) CD spectra of d(CGC[m^{8}G]CG) (oligomer **2**, 0.15 mM base concentration) in 5 mM Na cacodylate buffer, pH 7.0, under various temperatures.

of 24 transients. The starting model was constructed by MidasPlus (UCSF). Forty cycles of refinement of the starting model were then carried out by the sequence of procedures comprising the SPEDREF package (13). This includes a full matrix relaxation calculation of the NOEs for the model with comparison of the experimental and simulated spectra to deconvolute overlapped areas of the spectra. Minimization of the residual errors within the program X-PLOR (15) was then performed using conjugate gradient minimization of the NOE-derived force springs together with the chemical force field. A refined structure was obtained with the NMR *R* factor ($\Sigma|N_o - N_c|/\Sigma N_o$, where N_o and N_c are the experimental and calculated NOE cross-peak intensities respectively) is 15.2%. The optimal rotational correlation time was determined to be 6 ns using the procedure described before (13). The coordinates and related molecular constraints of the refined structure have been deposited in the Brookhaven Protein Databank (identifiers ITNE and RITNEMR).

Analysis of the thermodynamic data

Circular dichroism (CD) spectra were recorded on a Jasco J-700 spectrophotometer equipped with a Peltier temperature controller. CD spectra of oligonuclotide solutions (0.1 mM duplex in 30 mM phosphate, pH 7.0) were recorded using a 1 cm path length cell. CD spectra at different temperatures were recorded at intervals of 5°C with a 1 min equilibration period.

Thermal denaturation profiles were obtained with a Jasco V-550 spectrophotometer equipped with a Peltier temperature controller. Absorbance of the samples was monitored at 260 nm from 0 to

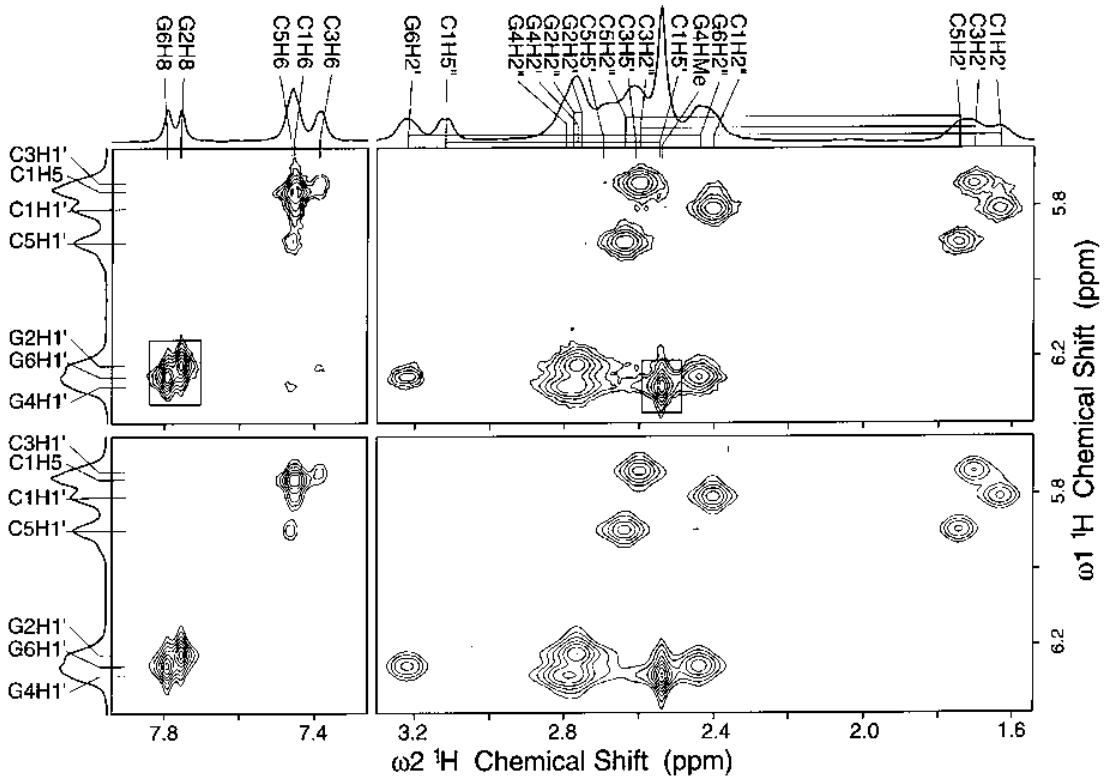

Figure 2. The experimental and simulated 2D NOESY spectra of the $H^{1'}$–aromatic/$H^{2'}$/methyl region of the native d(CGC[m^8G]CG)$_2$ at 2°C. The refined NMR *R* factor was 15.2%. Note the strong $GH^{1'}$–GH^8/GMe^8 cross-peaks due to the *syn* conformation. C_3H^5 and C_5H^5 are unusually upfield shifted to 5.07 and 5.20 p.p.m. (not shown in this figure) respectively, due to the ring current influence of the guanine bases on the 3′-side in Z-DNA.

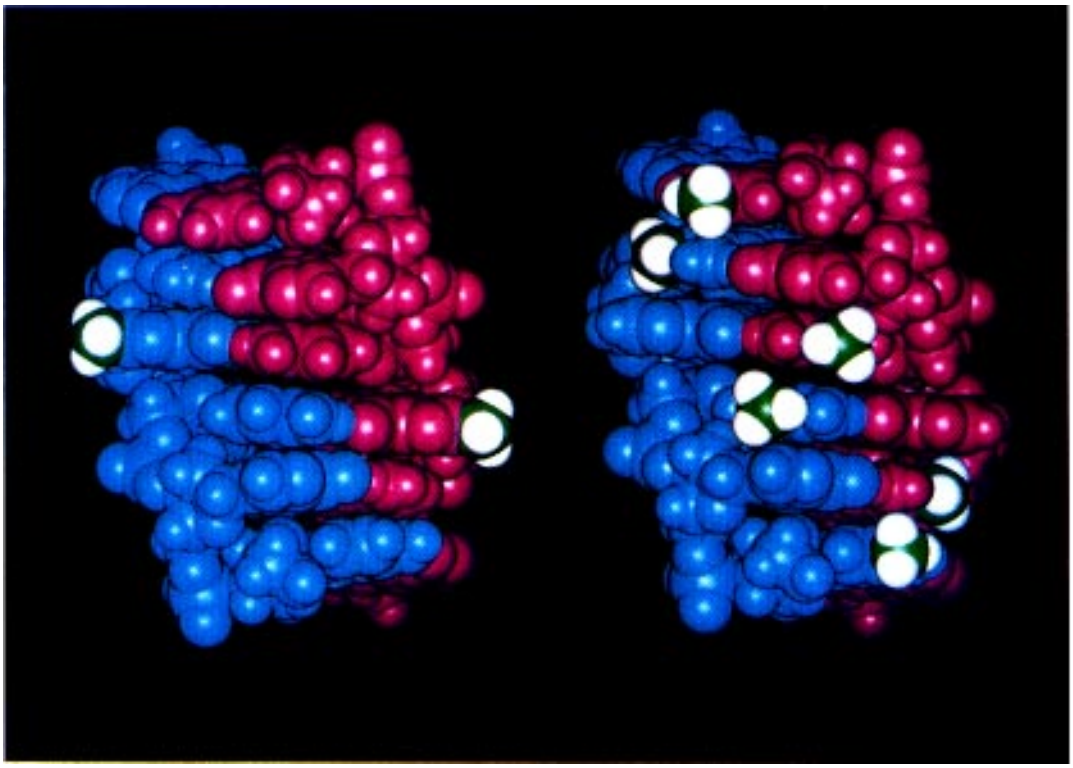

Figure 3. The refined model for d(CGC[m^8G]CG)$_2$ Z-DNA structure (left) and the model of d(m^5C-G)$_3$ (right).

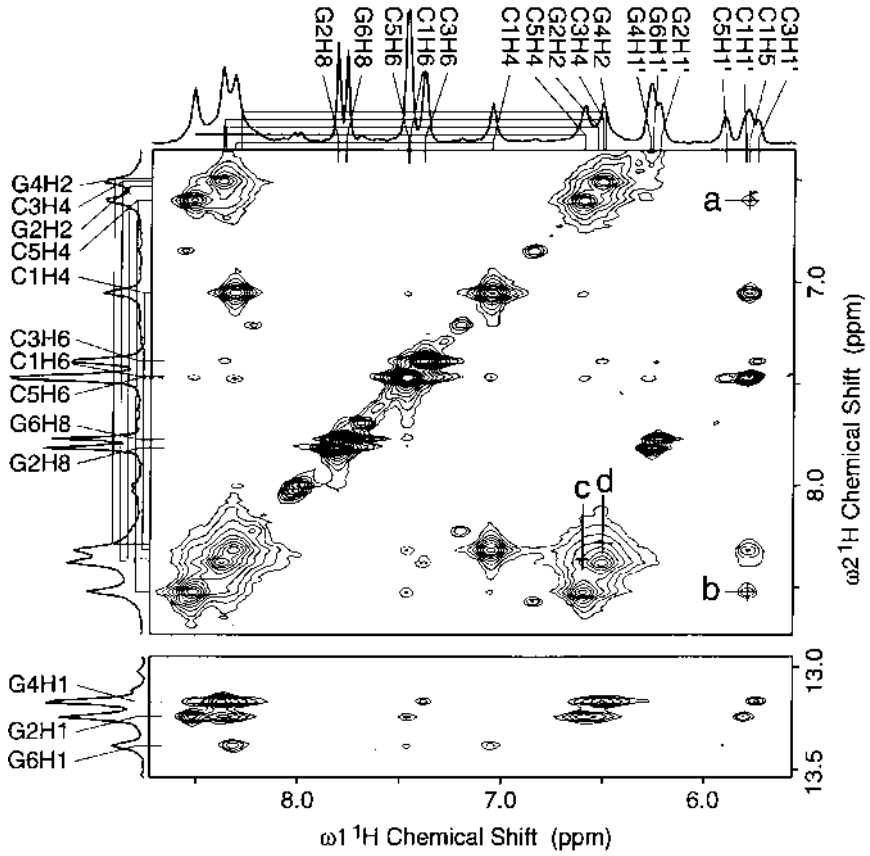

Figure 4. The exchangeable proton 2D NOESY spectra of the $H^{1'}$–aromatic region of d(CGC[m^8G]CG)$_2$ at 2°C. There are clear NOE cross-peaks (peaks a and b) between C_1H^4 amino protons and C_5H^5 protons from the opposite strand. Such cross-peaks can only happen in a Z-DNA structure. Note that the guanine N^2 amino geminal protons have a broad cross-peak (peaks c and d).

80°C with a heating rate of 1°C/min. Experiments with a heating rate of 0.5°C/min gave the same results, suggesting that thermodynamic equilibrium had been achieved. The data were normalized to percent denaturation. A linear least squares analysis of the data gave a slope of transition and the *y*-intercept, from which the melting temperature was calculated.

The proportions of Z, B and single-stranded (SS) forms in a m^8G-containing oligomer were determined by means of CD and UV spectroscopy as reported (16). Since the molar extinction coefficients of the B and Z forms of the hexamer were found to be approximately the same, the proportions of SS relative to that of B and Z at each temperature were estimated by UV melting experiments at 260 nm. The relative ratio of the amount of B and Z was determined by the CD ellipticity at 295 nm and by NMR (*vide infra*).

RESULTS AND DISCUSSION

Although theoretical calculations suggested that methylation at the guanine C^8 position greatly stabilizes the Z conformation by favoring the *syn* glycosyl conformation (17), such a property associated with m^8G-modified DNA has not been examined experimentally. While introduction of the bulky bromine atom at the C^8 position has been used previously (10,11), the brominated DNA suffered the problem of chemical/photochemical instability. It would be desirable to use the more stable m^8G in DNA to investigate the molecular basis of a variety of Z conformation-specific reactions at the oligonucleotide level.

The CD spectra of d(CGC[m^8G]CG)$_2$ (**2**) at different salt concentrations are shown in Figure 1A at 10°C. The hexamer in a 50 mM NaCl solution has the characteristic CD spectrum of Z-DNA. Without added salt it is in the SS form, as judged by UV and CD spectroscopy, and is converted to the Z form by increasing salt concentration, with a mid-point at 4.5 mM NaCl. Since the respective mid-point NaCl concentrations for d(CGCGCG)$_2$ and d(m^5CGCGm^5CG)$_2$ are 2.6 M (18) and 2.0 M (7), it is evident that C^8-methylation of guanine greatly stabilizes the Z conformation.

NMR refinement of Z-DNA

In order to unequivocally demonstrate that the structure of d(CGC[m^8G]CG)$_2$ (**2**) at 30 mM salt concentration is Z-DNA, NOE-restrained refinement has been carried out. 2D NOESY and TOCSY in D_2O were used to assign the resonances of all non-exchangeable protons. Since the structure is expected to be Z-DNA, as judged from the CD spectrum, the usual sequential assignment procedure would not be applicable. Indeed, the aromatic–$H^{1'}$ and m^8G_4 methyl–$H^{1'}$ cross-peak region of the 2D NOESY spectrum (Fig. 2) showed only strong intranucleotide $G_2H^{1'}$–G_2H^8, $G_4H^{1'}$–G_4Me and $G_6H^{1'}$–G_6H^8 cross-peaks, indicative of the *syn* conformation of guanine residues. As has been noted before (6,19), there is no internucleotide connectivity in Z-DNA, in contrast to that in right-handed B-DNA. The assignment was subsequently extended to the aromatic–$H^{2'}$/$H^{2''}$ region and finally to all regions of the spectrum. The TOCSY data supported the assignment (data not shown).

Table 1. Chemical shifts (p.p.m.) for d(CGCm^{8}GCG)$_2$ at 2°C

	H5/Me	H8/6	H1′	H2′	H2″	H3′	H4′	H5′	H5″	H1	H2a/4a	H2b/4b
C1	5.76	7.45	5.81	1.63	2.40	4.57	3.65	2.54	3.12		8.31	7.05
G2		7.75	6.22	2.77	2.77	5.07	4.19	4.14	4.11	13.24	8.36	6.52
C3	5.07	7.39	5.74	1.70	2.60	4.80	3.80	2.61	3.78		8.38	6.51
m^{8}G4	2.54		6.28	2.79	2.79	5.00	4.19	4.16	4.13	13.17	8.26	6.49
C5	5.20	7.47	5.90	1.75	2.64	4.82	3.90	2.69	3.82		8.52	6.60
G6		7.79	6.25	3.22	2.44	4.85	4.20	4.33	4.11	13.38	na	na

H2a and H4a are base-pair hydrogen bonded amino protons, H2b and H4b are not.

Table 2. Thermodynamic parameters for Z–B transition of d(CGC[m^{8}G]CG)$_2$ (**2**) and d(CGCGCG)$_2$ at 2.6 M NaCl[a]

Oligonucleotide	ΔG^{297K} (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (eu)
d(CGCm^{8}GCG)$_2$ (**2**)	0.81	14.5 ± 1.2	46.2 ± 2.8
d(CGCGCG)$_2$	–0.80	11.8 ± 0.6	42.6 ± 2.2

[a]Z–B conformational transition was analyzed by a two-state model from the data below 27°C. Thermodynamic parameters were obtained by plotting In(fraction Z/fraction B) versus 1/T.

The chemical shifts of all resonances are tabulated in Table 1. Note that all cytidine H$^{2'}$ and H$^{5'}$ resonances are unusually upfield (~1.7 and 2.6 p.p.m. respectively), analogous to those seen before (6,19). The upfield shifts are due to the orientation of the sugar moiety of the dC nucleotide in Z-DNA, which places the H$^{2'}$ and H$^{5'}$ protons directly under the ring current of the neighboring 5′- and 3′-dG guanine bases respectively.

All our data point to the inevitable conclusion that d(CGC[m^{8}G]CG)$_2$ has a structure consistent with Z-DNA. We constructed a model of d(CGC[m^{8}G]CG)$_2$ by appropriate methylation of the Z-DNA d(CGCGCG)$_2$ crystal structure (20) and subjected it to a combined SPEDREF (13) and NOE-constrained refinement (15). We measured 710 NOE integrals as the input for the NOE-restrained refinement. The refined structure, which has an NMR *R* factor of 15.2%, is shown in Figure 3. The NOE-refined structure is very similar to the d(CGCGCG)$_2$ Z-DNA structure determined by X-ray crystallography (20). The r.m.s. difference between the two structures is only 0.6 Å. The cytidine residues are in the *anti*/C2′-*endo* conformation, whereas the guanosine residues are in the *syn*/C3′-*endo* conformation (except for the 3′-terminal guanosines, which have a mixed C2′-*endo*/C3′-*endo* sugar pucker). In the m^{8}G-modified Z-DNA structure the hydrophobic C^8-methyl groups are located in the periphery of the helix and prominently exposed to the solvent region. In contrast, in the m^{5}C-modified Z-DNA structure the C^5-methyl groups form hydrophobic patches in the small recessed area of the concave 'major groove' (Fig. 3). The simulated NOESY spectra based on the refined model agree with the observed data (Fig. 2). To the best of our knowledge this is the first example of a refined structure of Z-form DNA by NMR under physiological salt conditions without added organic solvent or divalent cation.

Dynamics of Z-DNA

Z-DNA has been shown to have unusual rigidity (2). The measured T_1 relaxation inversion recovery time (T_1IR) of 2.7 s for the d(CGC[m^{8}G]CG)$_2$ helix supports this notion. For a B-DNA hexamer the averaged T_1IR is ~1.7 s. The stiffness of the Z-DNA double helix is also reflected in the remarkably slow exchange rate of its various exchangeable protons, including the G imino, G amino and C amino protons. It has been shown that the G imino and C amino protons exchange with water in 30 and 50 min respectively, whereas the G amino protons exchange in 330 min, at 5°C and pH 7 (21). Our ability to obtain a stable Z-DNA structure under physiological conditions affords a unique opportunity to investigate the behavior of the exchangeable protons.

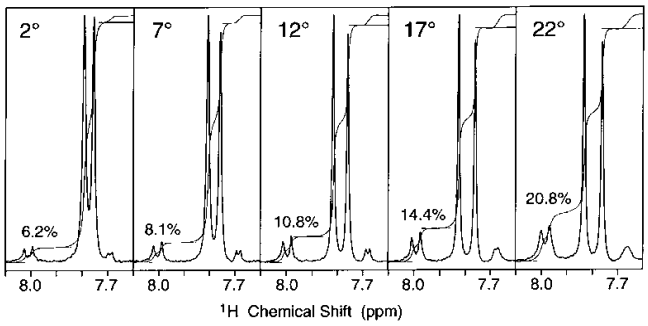

Figure 5. Proton 1D NMR spectra showing the temperature-dependent equilibrium of the B–Z transition as monitored by the G$_2$H^8 (7.75 p.p.m. at 2°C) and G$_6$H^8 (7.79 p.p.m. at 2°C) protons. The population of the B form increases from 6.2% at 2°C to 20.8% at 22°C.

The exchangeable proton NMR spectrum in H_2O (Fig. 4) revealed three clear imino proton resonances at 13.17 (G_4), 13.24 (G_2) and 13.38 (G_6) p.p.m., suggesting Watson–Crick-type base pairs. The assignment was aided by the 2D NOESY cross-peaks between the imino protons and other protons (Fig. 4). The cross-peaks associated with the exchangeable protons are again consistent with Z-DNA. For example, we note that C_5-NH^4 amino protons have cross-peaks (peaks a and b) to the C_1-H^5 proton. Such cross-peaks can only happen between the two interstrand cytosines in the C_1pG_2:C_5pG_6 step of the Z-DNA hexamer, due to its extreme sheared base pair stacking pattern.

Table 3. Midpoint NaCl contentration in B–Z transition of various 8-methylguanine-containing oligonucleotides

Oligonucleotide[a]	Number of residue			NaCl (mM)
	m^8G	*syn*-A[b]	*syn*-T[c]	
d(CGCG*CG)$_2$ (**2**)	2	0	0	30[d]
d(CGCGCG)$_2$	0	0	0	2600[e]
d(m^5CGCGm^5CG)$_2$	0	0	0	2000[f]
d[(Gm^5C)$_4$A^{Br}U(Gm^5C)$_4$]$_2$ (**3**)	0	0	0	–
d(CG*CATG*CG)$_2$ (**4**)	4	2	0	45[d]
d(TG*CATG*CA)$_2$ (**5**)	4	4	0	470
d(CG*CATG*TG) (**6**) d(GCGTACAC)	2	3	0	2450
d(CG*CTCG*CG) (**7**) d(GCG*AGCG*C)	4	0	1	120

[a]G*, 8-methyl-2′deoxyguanosine; m^5C, 5-methyldeoxycytidine; [b]*syn*-A conformation; [c]*syn*-T conformation; [d]transition from single strand; [e]reference 18; [f]reference 4. The data were taken at 10°C.

Note that cross-peaks between the geminal G amino protons are also observed, despite their broad resonances. The chemical shifts of the G amino protons (~8.4 and ~6.5 p.p.m.) are the same as those observed in (C·G)$_{12}$ at 5°C (21). The fact that we observed two separate resonances for each G amino group suggests that the rotation around the C^2–N^2 bond of G in Z-DNA is slower than B-DNA on the NMR time scale. This is likely due to the *syn*-G conformation, which allows the N^2 amino group to hydrogen bond with the phosphate oxygen either directly or through bridging water molecules.

Kochoyan *et al.* (21) have determined that the base pair lifetime for Z-DNA is ~3 s at 5°C. We have measured the half-time ($t_{½}$) of the exchange process for the imino protons and obtained values of 60, 300 and 700 ms respectively for G_6, G_4 and G_2. It is clear that at the hexamer level the exchange rate of imino protons in a solution containing only 40 mM phosphate buffer, pH 7.0, is significantly faster than the base pair lifetime.

Thermodynamic properties

The effect of m^8G substitution on the thermodynamic stability of the Z conformation was examined by measuring the proportions of the Z, B and SS forms at various temperatures. Figure 1B shows the CD spectra of d(CGC[m^8G]CCG)$_2$ (**2**) in 2.6 M NaCl solution at various temperatures. At 2°C it is nearly 100% Z-DNA. The proportion of B increased with increasing temperature. For comparison, d(CGCGCG)$_2$ under the same salt conditions consisted of a 1:1 mixture of B and Z. The proportions of Z, B and SS for a m^8G-containing oligomer were determined at various temperatures by means of CD and UV spectroscopy as previously reported (16). A similar temperature-dependent B:Z equilibrium has also been observed for d(CGC[m^8G]CCG)$_2$ at 30 mM salt concentration by NMR spectroscopy (Fig. 5).

The results for **2** and d(CGCGCG) at 2.6 M NaCl as a function of temperature are shown in Figure 6a and b. The thermodynamic parameters for the Z–B transition of the hexamer were determined from the data below 27°C, where >97% of the hexamers are in either the Z or B form. A van't Hoff plot for the Z–B transition of d(CGCGCG)$_2$ and d(CGC[m^8G]CG)$_2$ and the resulting ΔH and ΔS are shown in Figure 6c and Table 2 respectively. It is evident that the large stabilization of the Z conformation by introducing a methyl group at the guanine C8 position is enthalpic in origin and that the methyl substitution stabilizes the Z form by at least 0.8 kcal/mol, which roughly corresponds to half of the reported free energy (~1–2

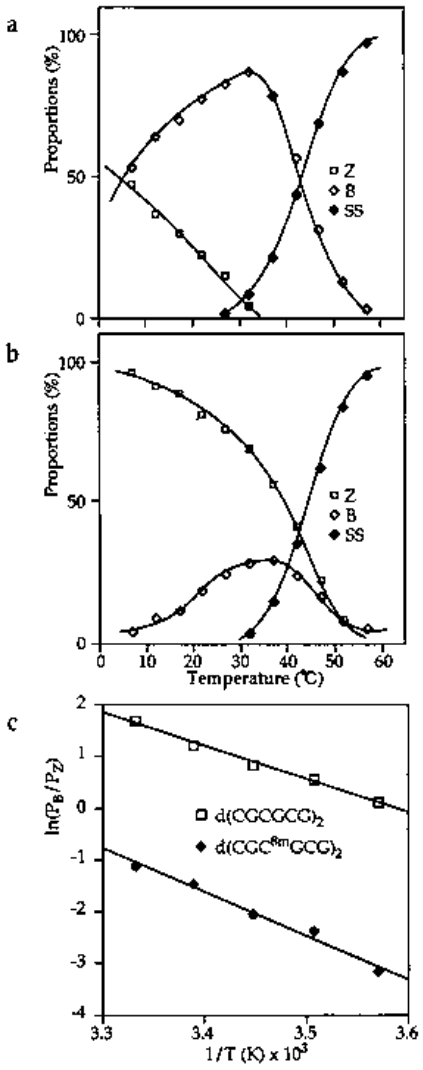

Figure 6. Proportions of Z, B and SS conformations of (**a**) d(CGCGCG) and (**b**) d(CGC[m^8G]CG) (**2**) as a function of temperature. Sample solutions contained 0.15 mM hexanucleotide (base concentration) in 2.6 M NaCl, 5 mM Na cacodylate buffer, pH 7.0. Proportions of Z, B and SS were obtained by a combination of UV and CD spectroscopy. (**c**) van't Hoff plot for the Z–B conformational transition of d(CGCGCG) and d(CGC[m^8G]CG) (**2**) obtained from the optical data and the NMR data from Figure 5.

kcal/mol) (22–24) required to shift the equilibrium to the *syn* conformation of C^8-substituted deoxyguanosines. A similar extent of stabilization of *syn* conformation by incorporating 8-bromodeoxyguanosine into oligomers has recently been reported for a G quartet structure (25).

When A·T base pairs are inserted into alternating $(C·G)_n$ sequences the B–Z transition is known to become more difficult (3,4,26). For instance, the 5-methylcytosine (m^5C)-containing octadecamer $d(G\text{-}m^5C)_4A^{Br}U(G\text{-}m^5C)_4$ (**3**) was found to retain the typical B form even at 4.0 M NaCl. Thus we examined the properties of various types of m^8G-containing oligomers having an A·T base pair in order to evaluate Z form stabilization induced by incorporation of m^8G (Table 3). In general the Z conformation was further stabilized by increasing the number of m^8G incorporated and destabilized by incorporating *syn*-A and *syn*-T. The CD spectrum of $d(C[m^8G]CAT[m^8G]CG)_2$ (**4**) indicates that this oligomer is converted from coil to Z with a mid-point at 45 mM NaCl. Oligomer **5**, which is obtained by replacing the terminal G·C base pairs of **4** with A·T base pairs, maintained the Z conformation with a mid-point at 470 mM NaCl. The incorporation of m^8G into only one strand is also capable of stabilizing the Z conformation considerably (oligomer **6**). A non-alternating pyrimidine–purine sequence has been shown to destabilize the Z conformation due to the energetically disfavored *syn* conformation of pyrimidine nucleosides (3,4). One of the central G·C base pairs of $d(CGCGCGCG)_2$ can be replaced by a T·A base pair without significantly increasing the mid-point NaCl concentration, if the duplex incorporates two m^8G into each strand (oligomer **7**). Such a low salt concentration requirement of 120 mM for an imperfect Z-DNA (out-of-alternation pyrimidine–purine sequence) is remarkable. Our results suggest that we can now study many heretofore inaccessible DNA conformations involving Z-DNA, e.g. the B–Z junction and the Z–Z junction. Such experiments are under way.

Conclusion

The substitution of a methyl group at the guanine C^8 position dramatically stabilizes the Z conformation of short oligonucleotides with a variety of base sequences. Some of these m^8G-modified oligomers exist as a stable Z form under physiological salt conditions without added organic solvent or divalent metal (3–7). While significant information on specific chemical reactions for DNA local structures has been accumulated during the past several years (27–30), considerably less is known about the origin of these specificities. Incorporation of the m^8G moiety into DNA oligomers could be a powerful tool to examine the molecular basis for many types of Z conformation-specific reactions at the oligomer level under physiological conditions.

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