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6-Thioguanine alters the structure and stability of duplex DNA and inhibits quadruplex DNA formation

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ABSTRACT

The ability to chemically synthesize biomolecules has opened up the opportunity to observe changes in structure and activity that occur upon single atom substitution. In favorable cases this can provide information about the roles of individual atoms. The substitution of 6-thioguanine (6SG) for guanine is a potentially very useful single atom substitution as 6SG has optical, photocrosslinking, metal ion binding and other properties of potential utility. In addition, 6-mercaptopurine is a clinically important pro-drug that is activated by conversion into 6SG by cells. The results presented here indicate that the presence of 6SG blocks the formation of quadruplex DNA. The presence of 6SG alters the structure and lowers the thermal stability of duplex DNA, but duplex DNA can be formed in the presence of 6SG. These results indicate that some of the cytotoxic activity of 6SG may be due to disruption of the quadruplex structures formed by telomere and other DNAs. This additional mode of action is consistent with the delayed onset of cytotoxicity.

INTRODUCTION

Site-specific replacement of amino acid and nucleic acid residues has been widely used to investigate the roles of individual residues in structure and function. However, substitution of an entire residue can introduce too many simultaneous changes to allow dissecting out of the roles of individual atoms. The chemical synthesis of biomolecules allows the substitution of single atoms so as to probe the structural and functional contributions on an atom by atom basis.

A single atom substitution gives rise to 6-thioguanine (6SG), whose structure is shown in Figure 1. 6SG is of interest for many reasons. It is a widely used cytotoxic agent of clinical significance (1–6). The metal binding properties of thio and oxo guanines are different (7). 6SG can also be used for crosslinking studies and 6SG absorbs at wavelengths longer than that of normal DNA and, hence, can also act as a site-specific optical probe (8–10). The chemical shifts of 6SG and guanine are sufficiently different to potentially aid the making of NMR assignments. Thus, we decided to examine the effects of 6SG substitution on the structures and stabilities of duplex and quadruplex DNAs. The original goal was to use 6SG substitution as an aid to solving some challenging structural questions about quadruplex DNAs via the combined use of NMR and optical methods. However, the results presented here show that the effects of 6SG substitution on duplex and quadruplex DNAs indicate that 6SG is not a conservative substitution.

6-Mercaptopurine (6MP) and other thioguanines are widely prescribed for maintenance therapy of acute lymphoblastic leukemia and for inflammatory bowel disease that is unresponsive to steroids (1,2), as well as for gliomas (3). 6MP is an inactive pro-drug that requires absorption, cellular uptake and intracellular anabolism to 6SG for cytotoxic activity to occur (1,5,6). The incorporation of 6SG nucleotides into DNA and RNA by polymerases can lead to cell death (1,11). 6SG is anabolized to the same intracellular metabolites as 6MP, suggesting the therapeutic equivalence of 6MP and 6SG (1,4,11).

The cytotoxicity of 6SG has a delayed onset that begins after one round of replication (1,11–14). The cytotoxicity appears to depend upon the incorporation of 6SG into DNA during replication. Methylation of the thio group of 6SG in DNA gives 6SMeG (11,14–17). Miscoding of 6SG during replication gives rise to T opposite the modified base and 6SMeG codes about equally well for T and for C (14,17). The post-replicative mismatch repair system (18–21) cuts the incorrect DNA strand at GATC sequences in the presence of 6SMeG-T base pairs (11). The cytotoxic effects of 6SG, cisplatin, DNA methylation and several other types of damaged DNA are partially due to this nicking of DNA by the mismatch repair system (11,18–21). Cells deficient in mismatch repair can exhibit partial resistance to 6SG cytotoxicity (22,23).

The *S*-methylation of thiopurines, including 6SG, is catalyzed by thiopurine *S*-methyltransferase (TPMT), which exhibits genetic polymorphism (16). About 1 in 300 people inherit TPMT deficiency as an autosomal recessive trait. A standard dose of thiopurine to a TPMT-deficient patient can induce excessive thionucleotide levels in hematopoietic tissues, leading to severe or even fatal toxicity. Low doses of mercaptopurines have been used to successfully treat TPMT-deficient patients (16).

The results of calculations have suggested that the replacement of guanine by 6SG or replacement of cytosine by thiocytosine leads to a decrease in the stability of double-helical DNA since the thio group is a poorer hydrogen bond acceptor than the normal carbonyl (15). The incorporation of 6SG into DNAs has been found to alter the triplex–quadruplex equilibrium in favor of triplex formation (24–26). 6SG has been suggested to reduce the stability of quadruplex structures due to the

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increased radius and decreased electronegativity of the sulfur, which would destabilize the Hoogsteen hydrogen bonding of guanine quartets relative to Watson–Crick pairing (24,25). The decreased electronegativity, due to the presence of the thio group, may also lead to weaker interactions with cations and water.

6SG can be used as a selective photocrosslinking agent since the 6SG absorbs at longer wavelengths, with a maximum near 340 nm, than most proteins or unmodified DNA (8–10,27). The incorporation of 6SG has allowed DNA–protein crosslinking efficiencies of up to 25% to be obtained (27). 6SG has also been shown to be reactive to organomercury(II) complexes (28). 6SG coordinates through the 6-thio group and a deprotonated N-7 as a bidentate ligand of organomercury(II). 6SG and unmodified DNAs may have different interactions with monovalent and other ions as thio is a ligand for 'soft' metals and oxygen for 'hard' (7). These thio–oxo differences in metal ion affinity have been widely exploited in the study of metals interacting with nucleic acid backbones by use of phosphorothioates (29–35).

To gain a further understanding of the effects of 6SG on the properties of DNAs we have examined a duplex DNA and two quadruplex DNAs that contain 6SG. The duplex DNA used is the familiar 'Dickerson duplex' that is formed from the self-complementary sequence d(CGCGAATTCGCG). The unmodified DNA has been compared to the equivalent DNA with a 6SG substitution at position 4 as shown in Figure 1. A 'chair' or 'edge' type quadruplex structure is formed by some DNA sequences, including that of the 15mer shown in Figure 1 (36–40). A 'basket' or 'crossover' type structure can be formed by sequences such as the 20mer shown in Figure 1 (36,41). The effects of 6SG substitution on both of these types of quadruplex structure have been examined. The presence of 6SG induces changes in the structures and stabilities of the duplex DNA but completely blocks the formation of quadruplex structure. The biological effects of 6SG may, in part, be due to its ability to disrupt the formation of quadruplex structures by telomere and other DNAs.

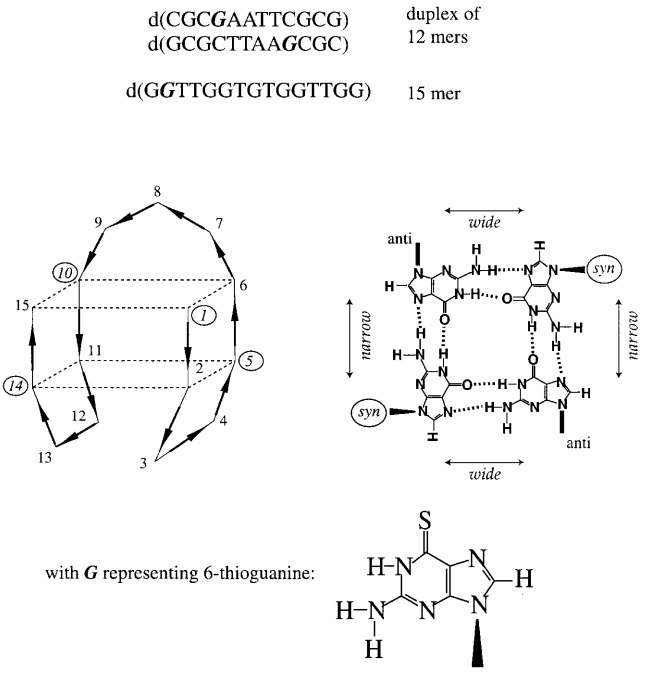

Figure 1. The structure of the 15mer d($G_1G_2T_3T_4G_5G_6T_7G_8T_9G_{10}G_{11}T_{12}$-$T_{13}G_{14}G_{15}$) is depicted along with the quartet pairing for this chair-type quadruplex DNA The sequence of the 12mer d($C_1G_2C_3G_4A_5A_6T_7T_8G_9C_{10}G_{11}C_{12}$), which forms a symmetric duplex, is also shown. The positions of 6SG substitution for both DNA contexts are also shown.

MATERIALS AND METHODS

The DNA samples d(CGCGAATTCGCG), d(CGC6SGAATTCGCG), d(GGTTGGTGTGGTTGG), d(G6SGTTGGTGTGGTTGG), d(GGTUTUGGUTUTGGUUTTGG) and d(G6SGTUTUGGUTUTGGUUTTGG) were obtained from Integrated DNA technologies Inc. (Coralville, IA) and were purified by HPLC. The 20mer sample was synthesized with dU residues so that the sequences of the three loops would be distinct to aid in assigning the spectra (41). The purity and integrity of the samples was checked by HPLC and the HPLC traces (not shown) indicated the presence of a single DNA for each of the samples. The proton NMR spectra of the 6SG and unmodified DNAs were obtained at temperatures above the melting temperature of the DNAs. Under these conditions the DNA is astructural and a comparison of the spectra of the 6SG and unmodified DNAs is consistent with only a single 6SG modification being present. The ^{31}P spectra of the samples were also obtained (see below) and these showed that there was no monoester present in the samples, demonstrating that the DNAs were intact strands.

The NMR samples of the 15mer and 6SG 15mer contained 100 A_{260} units of DNA in 500 µl of 140 mM NaCl, 5 mM KCl and 20 mM perdeuterated Tris buffer, pH 7.0, for a DNA concentration of 1.4 mM. One A_{260} unit is equivalent to an absorption of 1 at 260 nm in a 1 cm pathlength cell with the extinction coefficient of the unmodified 15mer (143 303). An additional set of 15mer samples was made up using 100 mM NaCl, 5 mM KCl, 10 mM $NaPO_4$ and 0.05 mM EDTA, pH 7.0, and there was no significant difference between the results obtained with Tris and those obtained with phosphate. The NMR samples, in 500 µl of 140 mM NaCl and 20 mM perdeuterated Tris buffer, pH 7.0, of the 20mer contained 160 A_{260} units, that of the 6SG 20mer 22 A_{260} units. The extinction coefficient of the unmodified 20mer was 181 200. The NMR samples of the 12mer and 6SG 12mer contained 100 A_{260} units of DNA in 500 µl of 100 mM NaCl, 5 mM KCl, 10 mM $NaPO_4$ and 0.05 mM EDTA, pH 7.0. Each sample was then dried, in the NMR tube, using N_2 gas and then dissolved with 500 µl of ^2H_2O. The pH was then checked and adjusted, if necessary, to 7.0. The DNA samples were annealed by placing the sample in an 80°C water bath, followed by allowing the entire water bath to slowly cool to room temperature.

The relative exchange rates of the H8 sites of 6SG and G deoxynucleosides were determined by heating a sample containing both the 6SG and G in 60% D_2O and 40% perdeuterated DMSO. Time points of 15 min and 2, 5 and 10 h at 80°C were obtained. The H8 of 6SG exchanges ~35% faster than the H8 of G under these conditions. This result showed that the H8

of 6SG would exchange slowly on the time scale of all of the NMR experiments carried out in this study.

All of the one-dimensional NMR experiments with the samples in ^2H_2O were carried out in a Varian 400 MHz Unity Plus spectrometer. One-dimensional spectra were obtained from 25 to 95°C at intervals of 10°C at 400 MHz and the experiments carried out on the samples in 95% H_2O/5% ^2H_2O used Watergate suppression of the water resonance (42). 1024 transients were collected at each temperature interval with a spectral width of 6000 and a delay time of 1 s.

One-dimensional experiments on the samples in 95% H_2O/5% ^2H_2O were carried out in a Varian Inova 500 MHz spectrometer using Watergate suppression of the water resonance (42). 4096 transients were used at 5°C with a spectral width of 20 000 Hz and a delay time of 1 s. The available probes for the 500 MHz spectrometer cannot be used above 45°C.

NOESY experiments of the duplex samples in ^2H_2O solutions were obtained at 500 MHz with the samples at 15°C with a mixing time of 250 ms and a delay time of 1 s. A spectral width of 5000 Hz was used in both dimensions with 128 transients acquired for 300 increments of t_1. A spectral width of 12 000 Hz was used in both dimensions with 96 transients acquired for 400 increments of t_1. Gaussian weighting functions were used in both dimensions.

The optical melting experiments were conducted using a Hitachi U-2000 UV spectrometer. Temperature control was maintained by a Lauda RC 3 Brinkman heater/cooler. Spectra were obtained from 25 to 85°C at 5°C intervals. A 15 min time period was allowed for equilibration of the sample at each temperature step. The scanning range was set from 410 to 190 nm and the absorbance was monitored at 340 and 260 nm with a scan rate of 400 nm/min. Each sample was composed of 0.5 A_{260} units of DNA in 1 ml of buffer with 140 mM NaCl, 20 mM perdeuterated Tris and 5 mM KCl, pH 7.

RESULTS AND DISCUSSION

A structurally conservative substitution will have effects on the chemical shifts, NOEs and other spectral properties of the residue in which the substitution is made and the residues that directly interact with it. Negligible effects will be observed on the spectral properties of residues that are distant from the site of substitution and only a modest effect on the thermal stability if the substitution is structurally conservative. Since 6SG is potentially quite useful in studying the structures and functions of nucleic acids, we have examined whether it is a structurally conservative substitution for dG.

NMR of 6SG-containing DNA

The effects of 6SG substitution on a duplex DNA were examined by comparing the NMR results obtained on unmodified and 6SG-containing DNAs. The one-dimensional 400 MHz spectra of the two dodecamers were obtained with the samples in 95% H_2O/5% ^2H_2O and these are shown together in Figure 2 to facilitate their comparison. The integrated intensity of the imino region indicates the number of base pairs. The intensities in the imino regions of the spectra in Figure 2 indicate that both of these DNAs form duplexes. The dA-dT base pairs are present in the case of both the conventional and 6SG-substituted duplex. The linewidths of the resonances of the imino protons of the 6SG sample indicate that base pairs have lifetimes

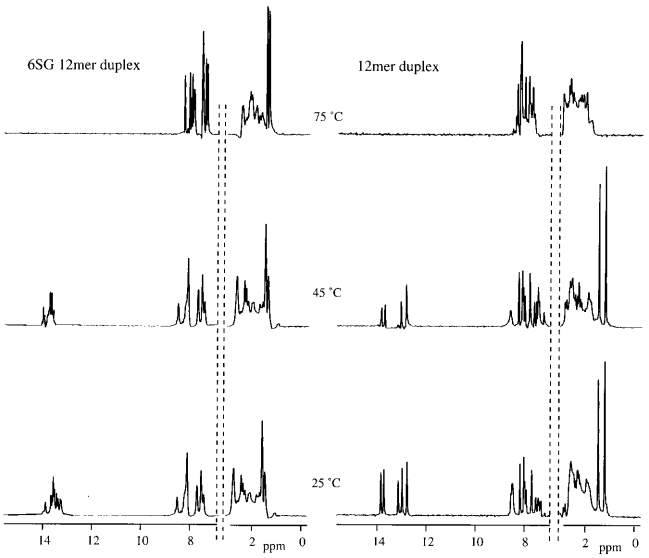

Figure 2. The 400 MHz proton spectra of the unmodified and 6SG-containing 12mer DNAs are shown as a function of temperature with the samples in 95% H_2O/5% ^2H_2O. The resonances of the imino protons are in the 12–14 p.p.m. region.

in excess of 100 ms at temperatures <50°C. There is no evidence for base pairing of the 6SG residues at any of the temperatures examined.

The chemical shifts of the imino protons are different for the unmodified and 6SG-containing DNAs. The linewidths of the AT imino protons are larger for the 6SG sample, indicating a higher exchange rate with solvent. Most of the imino protons of both samples are observable at 45°C, indicating slow exchange with solvent at this temperature. The spectra in Figure 2 also indicate differences in the chemical shifts in other regions. Additional spectra were obtained at 500 MHz with the samples at 5°C, with results analogous to those shown that were obtained at 15°C.

The region between 7 and 8.5 p.p.m. contains the signals of the non-exchangeable aromatic H8, H2 and H6 protons when the samples are in ^2H_2O. This region is shown in Figure 3 for the 6SG-containing and unmodified duplex samples. The results obtained below 45°C show that the chemical shifts of a number of sites have been altered by the 6SG substitution. The aromatic regions of the spectra of the 6SG and normal sample contain many differences, suggesting that the structural changes due to the presence of 6SG extend well beyond the site of substitution. The linewidths of many of the 6SG DNA resonances are greater than those of the unmodified sample at both 15 and at 45°C. The excess linewidth is evidence for conformational mobility or disorder of the 6SG-containing sample.

Similarly, it was found that the chemical shifts of the two methyl groups (data not shown) are ~0.5 p.p.m. further downfield in the 6SG case than in the unsubstituted DNA. This indicates that the T methyls are experiencing smaller ring current shifts in the 6SG case than in the G case, which suggests that these bases are less stacked in the 6SG sample.

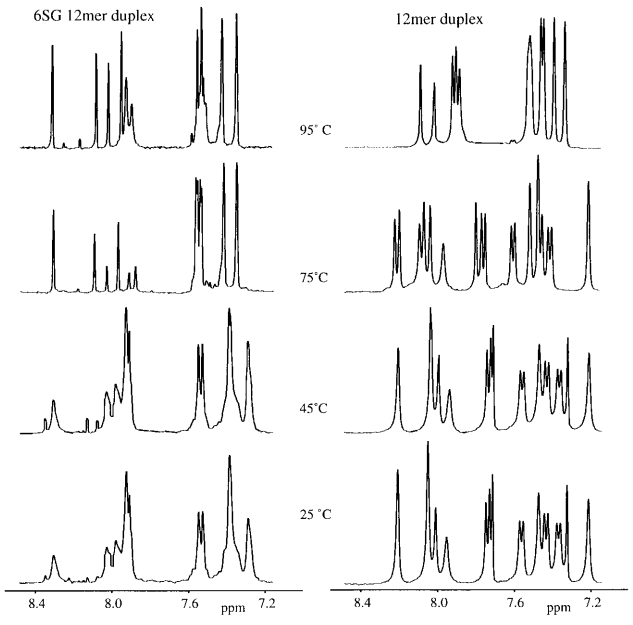

Figure 3. The 400 MHz aromatic region of the proton spectra of the unmodified and 6SG-containing 12mer DNAs are shown as a function of temperature.

The ^{31}P chemical shifts (data not shown) of the two samples also exhibit a number of differences. The proton chemical shifts were assigned and Table 1 contains the aromatic and H1' proton chemical shifts and some of the assignments are also given in Figure 4. The largest differences are of the order of 0.2 p.p.m. and are from the 6SG-C base pair and the adjacent base pairs. Chemical shift changes extend throughout the DNA. The chemical shifts in all of the spectral regions examined are indicative of structural changes associated with the introduction of 6SG that are not localized just to the single base pair in which the substitution was made.

To examine the extent and magnitude of the structural changes NOESY data were obtained on the two samples. Examination of the results shown in Figure 4 indicates that there are many differences between the NOESY maps of the two samples. Overall, the ratio of the crosspeak volumes of the inter-residue to intra-residue aromatic to H1' NOEs are smaller for the 6SG sample than for the unmodified one; these volumes are listed in Table 2. For example, there is a large change in the volume of the inter-residue H5–H1' NOE for the C3 adjacent to the 6SG site. This NOE volume is reduced by ~3-fold relative to the unmodified DNA. The volume of the intra-residue H8–H1' NOE of A5 decreases by about an order of magnitude upon 6SG substitution while that of A6 increases ~3-fold. These and the other NOE volume changes are indicative of a significant structural change induced by the presence of 6SG.

Stability effects of 6SG on duplex DNA

NMR and optical methods were used to examine the thermal stabilities of the two DNAs. The optical melting temperature of the unmodified DNA was found to be 53°C and that of the 6SG sample 38°C when melting was monitored at 260 nm. The hypochromicity of the unmodified DNA was 43% and that of the 6SG-containing duplex 15% and this DNA exhibited a non-cooperative melting profile. A prior report on the effects of 6SG on the thermal stability of duplex DNA observed a 7°C drop in the T_m of a non-palindromic 12mer duplex with a single 6SG-C pair relative to the corresponding duplex with a G-C pair when monitored at 260 nm (43). The melting of the 6SG-containing duplex sample was also monitored at 340 nm, since at this wavelength the absorption is due to the 6SG residue. The hypochromicity at 340 nm was found to be <5%. This small hypochromicity value indicates that the 6SG base is not well stacked under the conditions used for the optical experiments. The relatively small hypochromicity observed for the 6SG-containing duplex upon melting indicates that this DNA is partially disordered at all of the temperatures examined. The non-cooperative melting is consistent with the disorder being near the 6SG site, as deduced from the NMR results.

Table 1. Chemical shifts of the Dickerson duplex (DD) and 6SG-substituted duplex (6SG) at 15°C

Residue	H8/H6		H5/H2		H1'	
	DD	6SG	DD	6SG	DD	6SG
C1	7.75	7.55	5.97	6.01	5.81	5.87
G2	8.16	7.84			5.97	6.05
C3	7.46	7.34	5.44	5.40	5.61	5.58
G/6SG	8.07	7.97			5.55	5.68
A5	8.30	8.28	7.33	7.27	6.08	6.13
A6	8.31	8.29	7.75	7.82	6.21	6.24
T7	7.36	7.26			6.01	6.01
T8	7.56	7.35			6.19	6.10
C9	7.64	7.44	5.69	5.95	5.79	5.88
G10	8.10	7.87			5.92	6.01
C11	7.54	7.35	5.54	5.35	5.80	5.65
G12	8.15	7.88			6.24	6.12

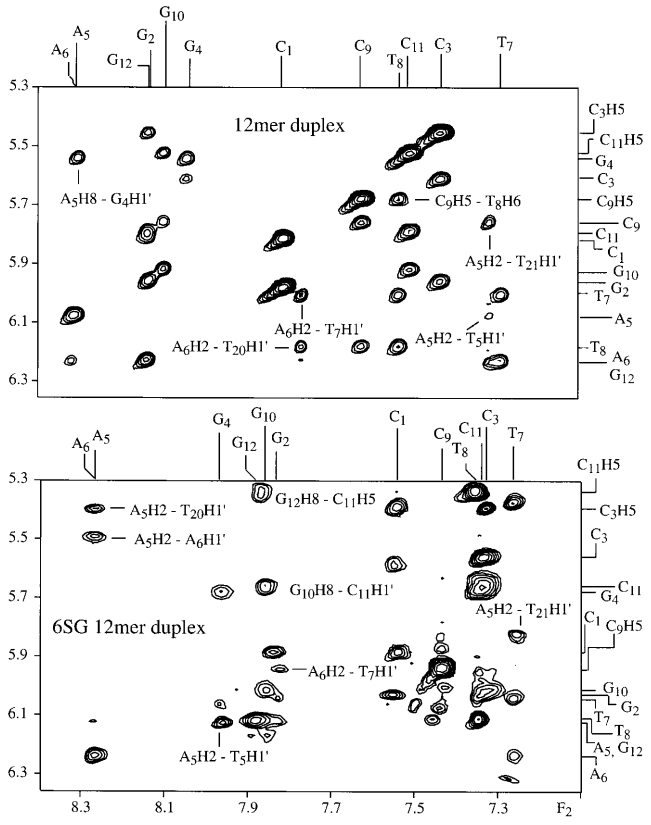

Figure 4. The 250 ms mixing time, 500 MHz NOESY spectra of the unmodified and 6SG 12mer duplex obtained with the samples at 15°C are shown. The assignments of the aromatic H8, H2 and H6 protons are indicated across the top and those of the H5 and H1' protons along the side. This region contains crosspeaks from both inter-residue and intra-residue connectivities. The two spectra have the same integrated volumes of the sum of the H5–H6 crosspeaks of the three, non-terminal cytosine residues.

The thermal stability of these DNAs was also examined at NMR concentrations. The aromatic regions of the spectra of the two samples are shown in Figure 3 as a function of temperature. These spectra show that the unmodified DNA has a melting temperature of >75°C whereas that of the 6SG DNA is ~55°C. The NMR melting temperature was taken as the lowest temperature at which the spectrum is that of the melted state. The spectra of the two samples in the melted state are essentially the same, with the exception of the resonances of the G and 6SG residues. The NMR melting temperatures are higher than the optical ones due to the higher concentrations used and the fact that duplex formation is a bimolecular reaction.

Summary of effects of 6SG on duplex DNA

The optical and NMR melting experiment results show that the introduction of 6SG considerably lowers the thermal stability of duplex DNA. The NOE results show that the introduction of 6SG perturbs the structure of the DNA both at the site of substitution and at the adjacent residues. The structural change is sufficiently large that 6SG cannot be considered to be a structurally conservative substitution in this case. The 6SG sample is too structurally disordered, even at low temperature, for a highly refined structure to be determined by NMR methods.

Table 2. NOE volumes of the aromatic–H1' cross-peaks of the Dickerson dodecamer (DD) and 6SG substituted duplex (6SG)

Residue	Intra-residue H8/H6–H1'		Inter-residue H8/H6–H1'	
	DD	6SG	DD	6SG
C1	355	121		
G2	139	76	152	78
C3	147	152	138	45
G/6SG	86	71	21	68
A5	159	12	81	52
A6	22	63	79	63
T7	86	72	144	64
T8	109	97	82	61
C9	124	68	100	71
G10	99	78	32	47
C11	184	137	67	43
G12	149	102	152	109

Both the intra-residue and inter-residue NOEs are given. The volumes of the two were normalized using the intra-residue H6–H5 NOEs of the dC residues.

Structural effects of 6SG on quadruplex-forming DNAs

The 15mer adopts an edge or chair type quadruplex structure, in the presence of potassium, as depicted in Figure 1 (37–40). The one-dimensional spectra of the unmodified and 6SG samples were obtained as a function of temperature and these are shown in Figure 5. The unmodified sample has a melting temperature, under these conditions, of ~45–50°C. The proton spectrum of the 6SG sample was found not to change appreciably over the temperature range 10–75°C, indicating that the sample did not form a quadruplex structure within this temperature range. The spectra of the modified and unmodified samples are similar only at temperatures above the melting point of the unmodified DNA. Addition of higher concentrations of potassium, up to 60 mM, did not induce quadruplex structure formation of the 6SG sample. ^{31}P NMR spectra of the modified and unmodified samples are shown in Figure 6 and these results also indicate that the 15mer does not form a quadruplex structure under any conditions investigated. The ^{31}P spectrum of the 6SG sample is similar to that of single-stranded, astructural DNA and the spectrum does not show a high dispersion of resonances in the spectrum of the unmodified DNA. No evidence was obtained by NMR or optical experiments for an ordered structure of the 6SG-modified 15mer under any conditions.

The 20mer forms a basket or crossover type structure, as depicted in Figure 1, in the presence of sodium alone and the addition of up to five potassium atoms per DNA does not change the structural type (41). The one-dimensional spectra of the unmodified and 6SG samples were obtained as a function of temperature and selected spectra are shown in Figure 7. The

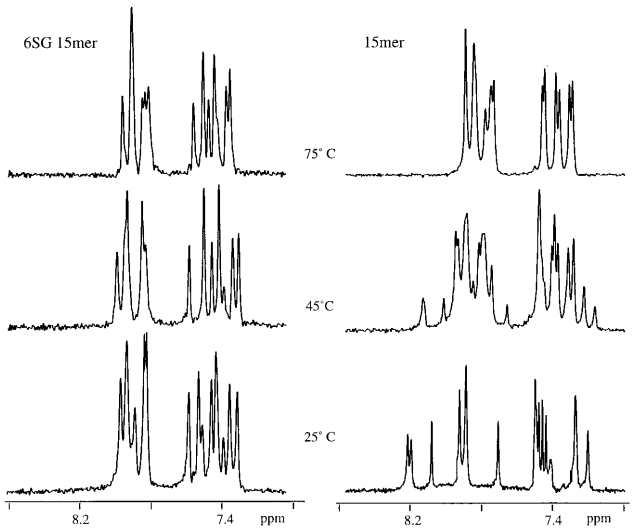

Figure 5. The aromatic region of the 400 MHz proton spectra of the unmodified and 6SG-containing 15mer DNAs are shown as a function of temperature.

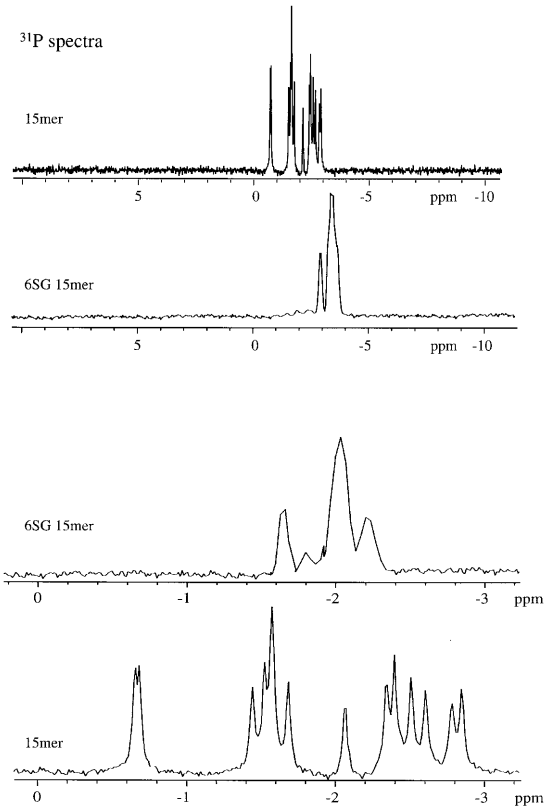

Figure 6. The 161 MHz ^{31}P spectra of the unmodified and 6SG-containing 15mer obtained with the samples at 15°C are shown. The spectra are shown with a wide spectral width to show the absence of any monoesters in the samples and with a narrow spectral width to highlight the differences between the spectra obtained from the two samples.

unmodified sample has a melting temperature, under these conditions, of ~70°C. The proton spectrum of the 6SG sample was found not to change appreciably over the temperature range 10–85°C, indicating that the sample did not form an ordered structure within this temperature range. No evidence for an ordered structure of the 6SG-modified 20mer was obtained by NMR experiments under any conditions.

Stability effects of 6SG on quadruplex-forming DNAs

The melting temperatures of the quadruplex structures formed by the 15mer and 20mer are approximately equivalent to those of DNA 8mer to 11mer duplexes. As noted above, each 6SG in a duplex DNA lowers the melting temperature of a dodecamer duplex by ~7°C. The effect of 6SG on the quadruplex DNAs is much greater than this. The presence of a single 6SG disrupts quadruplex formation in the two cases and the unmodified DNAs form quadruplexes which are thermally stable to >60°C. The larger effect of 6SG in the quadruplex context is likely due to the intrinsic cooperative nature of quadruplex structures. Each quartet needs the simultaneous interactions of all four members for stability. Disruption of the interactions between any two components can destabilize the entire quartet. In the two cases examined here, disruption of one of the two quartets leaves only one quartet, which does not provide sufficient stabilization. In a duplex DNA the 6SG directly destabilizes a single base pair. A single 6SG can apparently disrupt an entire quartet.

Summary and conclusions

Our original plan for these experiments was to characterize the effects of 6SG on the structures of duplex and quadruplex DNAs so that we could then exploit its novel spectroscopic properties in NMR and other studies. The perturbation due to the presence 6SG was expected to be modest and correspond to the loss of a single hydrogen bond at the site of substitution, as discussed in the Introduction. The results presented here clearly show that 6SG is a disruptive substitution of both Watson–Crick and Hoogsteen pairings. The thio group at position 6 may be disrupting interactions with water molecules and with cations, as well as being a weaker hydrogen bond acceptor than an oxo group.

Inosine can form duplex and quadruplex structures, though apparently not as well as guanosine. Inosine has been used as a replacement for guanosine to aid in making NMR assignments for quadruplex DNAs (44–46). However, in some cases inosine can be a structurally disruptive substitution (47). The results presented here show that the presence of 6SG induces a considerably larger perturbation than that of inosine, especially for the formation of quadruplex DNA.

Since a single 6SG can have large effects on the stability of duplex and quadruplex DNAs it may be the case that some of the biological effects of 6-mercaptopurines are due to destabilization of DNA and this may account for some of the cytotoxicity of mercaptopurines. The methylation at the 6-position may introduce further disruption of DNA structure. Cells that have been exposed to mercaptopurines will have, after one round of replication, some 6SG incorporation. Quadruplex DNAs may be formed by telomere (48–51), fragile X (52–54), other triplet repeat DNAs (54,55) and other DNAs. Thus, some of the cytotoxity of 6SG may be due to its ability to disrupt the

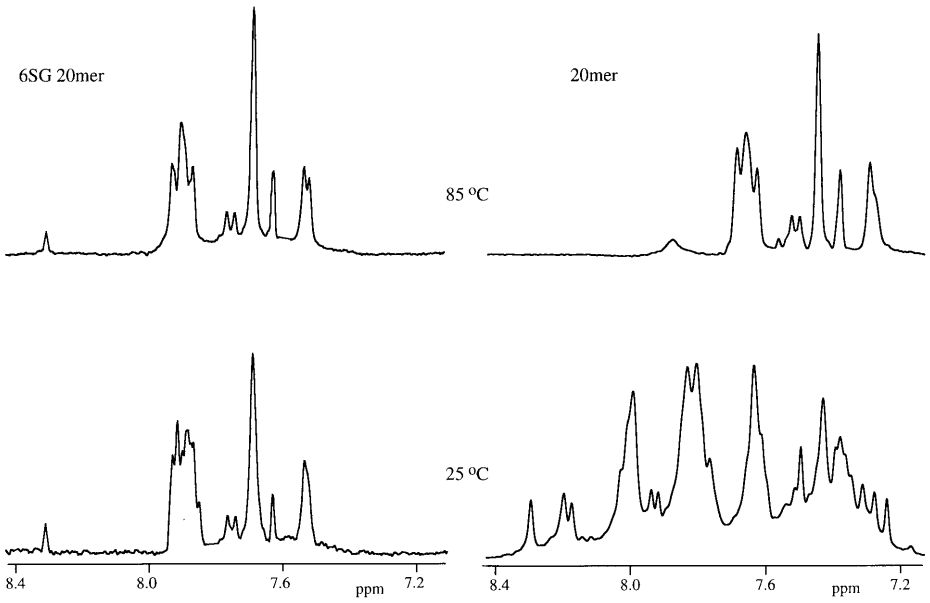

Figure 7. The aromatic region of the 400 MHz proton spectra of the unmodified and 6SG-containing 20mer DNAs are shown as a function of temperature.

quadruplex structures needed for the biological activities of these DNA. As 6SG appears to be a strong suppresser of quadruplex DNA structure its incorporation into quadruplex-forming sites may account for some of the cytotoxicity of 6SG, especially against proliferating cells. This mode of action is consistent with the delayed onset of mercaptopurine cytotoxicity as it depends on incorporation of 6SG into DNA.

In addition, the incorporation of 6SG into DNA could eliminate the blockage of replication that can be induced by the formation of quadruplex structures (56). The combination of the DNA polymerase stop assay with the use of the triphosphate of 6SG may allow a means to detect quadruplex formation by a wide range of sequences.

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