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O^6-Methylguanine and A·C and G·T Mismatches Cause Asymmetric Structural Defects in DNA That Are Affected by DNA Sequence†

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ABSTRACT: Mismatched and modified base pairs are central to questions of DNA mutation and repair. NMR and X-ray crystallography of mispairs indicate little to no local helical distortion, but these techniques are not sensitive to more global distortions of the DNA molecule. We used polyacrylamide gel electrophoresis and thermal denaturation to examine A·C, G·T, and O^6-methylG·T and O^6-methylG·C mismatches synthesized in place of either of two adjacent G·C base pairs in synthetic DNA duplexes. Substitution for G·C at either position decreased the stability of the duplex; O^6-methylguanine was more destabilizing in place of the 5′G than in place of the 3′G. Comparisons between polymers synthesized so that lesions occurred regularly spaced on the same side of the helix and polymers synthesized so that the lesions alternated from side to side on the helix showed that these lesions introduced helical distortion composed of (i) a symmetric frictional component, probably caused by localized bubble formation, and (ii) an asymmetric component indicative of a more global effect on the DNA molecule. Comparisons between these effects at the two adjacent positions show that the extent of structural perturbation depends on sequence context.

Two types of mutagenic lesions, namely, mismatched and modified base pairs, occur in DNA. Mismatched base pairs occur mainly as the products of natural processes: mismatched base pairs arise normally during genetic recombination (Bianchi & Radding, 1983), as incorporation errors during DNA replication (Loeb & Kunkel, 1982), and during the folding of single-strand DNAs. Mismatched base pairs also occur as the product of potentially detrimental processes such as the deamination of 5-methylcytosine in DNA to give G·T (Zell & Fritz, 1987) and repair of O^6-methylguanine–thymidine

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(O^6-meG·T) base pairs by alkyltransferase to give G·T (Toorchen et al., 1984).

Modified base pairs occur as the product of natural enzymatic processes, for example, N^6-meA in mismatch repair and 5-meC implicated in control of gene expression. Modified base pairs also occur as the product of environmental damage to DNA by both direct-acting chemicals and chemicals that require metabolic activation; a wide spectrum of DNA adducts is produced (Singer & Kusmierek, 1982). One of the most mutagenic of these lesions is O^6-meG.

O^6-meG and mismatched bases such as G·T and A·C are destabilizing; they decrease the T_m of DNA significantly (Gaffney et al., 1984; Gaffney & Jones, 1989). The structures of such modified and mismatched base pairs have been the subject of considerable investigation. The principal methods of study have been X-ray crystallography (Brown et al., 1985; Hunter et al., 1986) and NMR (Patel et al., 1986a,b; Kalnick et al., 1988a,b), with the general result that such base oppositions are not very disruptive to structure; they were all found to stack within the helix with only small perturbation to local structure. Yet, O^6-meG·C and O^6-meG·T are recognized in DNA by ABC excinuclease in vitro (Voigt et al., 1989); ABC excinuclease is a repair activity that apparently recognizes helical distortion rather than the lesion directly [reviewed by Sancar and Sancar (1988)]. In addition, mismatch repair locates A·C and G·T mismatches embedded in normally base-paired DNA [reviewed by Modrich (1987)].

To try and reconcile the NMR and crystallographic results with the ability of enzymatic activities to differentiate mismatched and methylated base pairs from normal base pairs, we synthesized oligonucleotide duplexes containing a GG doublet, but substituting in turn an O^6-meG·C, G·T, O^6-meG·T, and A·C base pair in place of one and then the other of the two adjacent G·C base pairs. The effects of substitutions on the stability of the duplex, as measured by T_m determinations, and on helical structure, as measured by changes of the electrophoretic mobility of the duplex in polyacrylamide gels, were determined and compared among the different substitutions. Polyacrylamide gel electrophoresis has proved to be quite sensitive to sequence-directed curvature of DNA structure, which is difficult to detect by X-ray crystallography and NMR (Marini et al., 1982; Wu & Crothers, 1984; Diekmann & Wang, 1985; Hagerman, 1985; Koo et al., 1986).

The results show that base substitutions at either position decrease the stability of the duplex relative to G·C. O^6-meG in place of G at the 5′G of the G doublet caused a greater decrease in stability than at the 3′G. Structural distortion differences, as measured by gel electrophoretic mobility changes, were quite evident under conditions where stability differences, as measured by T_m changes, were negligible. The structural distortions appear to be composed of a symmetric component that imparts a frictional drag on the polymer during gel electrophoresis and an asymmetric component that constitutes a more global effect on the conformation of the DNA. The extent of helix distortion depended on sequence context.

MATERIALS AND METHODS

Materials. 2′-Deoxynucleoside 5′-[2-cyanoethyl *N*,*N*-bis(dimethoxytrityl)phosphoramidites] were obtained from American Bionetics (Hayward, CA). Anhydrous solvents and other reagents for oligonucleotide synthesis were purchased from Applied Biosystems (Foster City, CA). T4 polynucleotide kinase, T4 DNA ligase, and exonuclease III were obtained from Promega Corp. (Madison, WI). P1 nuclease was obtained from Boehringer Mannheim (Indianapolis, IN); *Escherichia coli* alkaline phosphatase and deoxyribonucleosides were purchased froim Pharmacia Inc. (Piscataway, NJ). Adenosine triphosphate and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were obtained from Sigma Chemical Co. (St. Louis, MO). [γ-^{32}P]Adenosine triphosphate was purchased from New England Nuclear (Boston, MA).

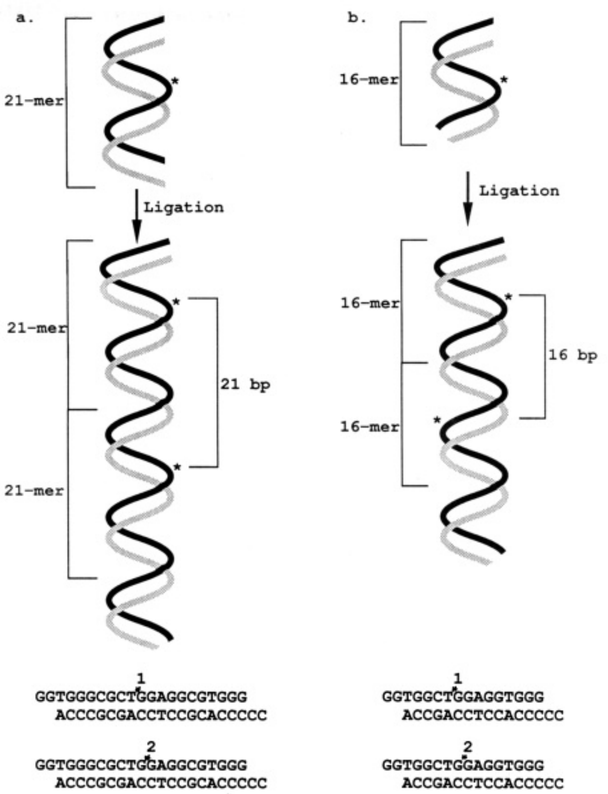

FIGURE 1: Ligation of 21-mers and 16-mers. Duplexes containing base substitutions at sites 1 and 2 were ligated to form polymers. (a) Polymer duplexes consisting of 21-mers. The lesions are located 21 bases apart on the same side of the helix. (b) Polymer consisting of 16-mers. The lesions are 16 bases apart on opposite sides of the helix. (*) Position of lesion; sequences of the 21-mers and 16-mers are shown at the bottom of the figure.

Oligonucleotide Synthesis and Purification. Oligonucleotides, with sequences as shown in Figure 1 and containing substitutions at either of two adjacent G's defined as sites 1 and 2 in Figure 1, were synthesized on an Applied Biosystems Model 380A DNA synthesizer. The purity of each phosphoramidite was reported by American Bionetics to be greater than 99% as analyzed by ^{31}P nuclear magnetic resonance and high-pressure liquid chromatography; efficiency of polymerization for the monomers was reported to be greater than 97%.

Following synthesis, 5′-tritylated oligonucleotides were cleaved from the resin and deprotected by exposure to 10% (v/v) DBU in anhydrous methanol for 5 days at 25 °C (Borowy-Borowski & Chambers, 1987). Detritylation was accomplished by exposure to 80% acetic acid for 20 min at 25 °C. Detritylated oligonucleotides were purified by elution from gel slices following electrophoresis on 20% denaturing polyacrylamide gels. The purity of the resulting oligonucleotides was determined by gel electrophoresis of 5′-^{32}P-labeled oligonucleotides.

The base content of each purified oligonucleotide was analyzed as described by Eadie et al. (1987). Briefly, oligonucleotides were digested with P1 nuclease for 3 h at 37 °C and with *E. coli* alkaline phosphatase for 4 h at 37 °C. The resulting nucleosides were separated by reverse-phase HPLC with monitoring of the UV absorbance. The products were quantitated by comparison with standard curves generated by injection of known amounts of authentic nucleoside standards. This method detected a single O^6-meG (±0.1) in a 50-base oligonucleotide.

Relative-Mobility Studies. Oligonucleotides were 5′-^{32}P labeled according to Maniatis et al. (1982) and annealed together in a mixture containing 20 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, and 100 mM NaCl by heating to 70 °C for 10 min followed by slow cooling to 25 °C. For the gel-mobility studies, oligonucleotide duplexes were ligated head-to-tail as follows: One microgram of 5′-labeled strand (top strand in Figure 1) was annealed with a 2-fold excess of its complement to ensure that all of the labeled oligonucleotide was in duplex form. The DNA was then ethanol precipitated, resuspended in 50 μL of 10 mM Tris-HCl, pH 7.5, containing 0.1 mM EDTA (TE), and desalted over Sephadex G-15 equilibrated with TE. Forty nanograms of desalted duplex was then ligated for 2 h at 4 °C in a volume of 100 μL containing 6.6 mM Tris-HCl, pH 7.6, 6 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, and 2 units of T4 DNA ligase. The product DNA was ethanol precipitated and resuspended in 15 μL of TE containing 5% glycerol, 0.5% bromophenol blue, and 0.5% xylene cyanol. The ligation products were electrophoresed on a nondenaturing 4% polyacrylamide gel. Following autoradiography, the relative mobilities of polymers were determined by dividing the distance of migration of the base-substituted polymer (measured on the autoradiogram) by the migration distance of the control polymer of the same length.

DNA Melting-Temperature Determinations. For T_m determinations 3 μg of each complementary strand was annealed and diluted to a volume of 1.2 mL containing 1.4 mM Tris-HCl, pH 7.6, 0.7 mM $MgCl_2$, 0.1 mM EDTA, and 25 mM NaCl. The DNA solution was placed in a Perkin-Elmer 320 UV-vis spectrophotometer (260 nm) connected to a digital temperature controller set to increase the temperature from 40 to 85 °C at a rate of 0.5 °C per minute. T_m values were determined from first-derivative plots of absorbance vs temperature as the temperature at which the increase in absorbance at 260 nm was greatest (inflection point of melting curve).

RESULTS

Mismatched and modified base pairs can alter the stability and structure of DNA. To study these alterations, we synthesized duplexes modified by substitution of G·T, O^6-meG·C, O^6-meG·T, or A·C in place of either of two adjacent G·C base pairs (Figure 1). Substitutions at these adjacent sites were studied to determine the effect of position on the structure of the DNA at the lesion. T_m values and relative gel electrophoretic mobilities of duplexes with lesions located on the same side and on alternating sides of the helix were studied to differentiate between localized and more global perturbations to helical structure.

Table I: T_m and Relative Electrophoretic Mobilities per Base Pair for Duplexes Containing G·C, G·T, O^6-meG·T, O^6-meG·C, and A·C Base Pairs

		21-mer			16-mer
site	base pair[a]	T_m (°C)[b]	ΔT_m	R_f/base pair ($\times 10^4$)[c]	R_f/base pair ($\times 10^4$)[c]
control	G·C	73.5 ± 0.3[a]		0.00	0.00
1	G·T	68.8 ± 0.4[b]	−4.7	<0.1	
1	G*·T	65.7 ± 0.4[c]	−7.8	−1.47	
1	G*·C	65.6 ± 0.4[c]	−7.9	−1.25	−0.61 (−0.47)[d]
1	A·C	66.2 ± 0.5[c,d]	−7.3	−3.08	−2.67 (−2.03)
2	G·T	68.7 ± 0.5[b]	−4.8	−0.55	
2	G*·T	66.9 ± 0.3[d]	−6.6	−0.74	
2	G*·C	68.4 ± 0.3[b]	−5.1	−1.13	
2	A·C	65.8 ± 0.0[c]	−7.7	−2.07	−1.15 (−0.88)

[a] G* is O^6-meG. [b] T_m values were determined as described under Materials and Methods. ΔT_m were calculated by subtracting the control (G·C) value from each observed value. Numbers shown are the mean ± standard deviation of at least two separate determinations. Differences between means were detected by a one-way ANOVA followed by a Tukey's *w* test (Ott, 1977). Means labeled with the same letters (a–d) are not significantly different ($P \leq 0.05$). [c] Values for relative electrophoretic mobility per base pair ($\times 10^4$ so that values are in the range of -1×10^{-4}) are the slopes of the plots in Figure 3. [d] Values in parentheses are corrected to the same density of lesions per base pair as in ligated 21-mers.

Effects of Base-Pair Substitutions on Relative Mobilities of Polymers. Gel electrophoresis of ligated duplexes has proven useful for detecting helical distortions caused by base damage (Husain et al., 1988; Hsieh & Griffith, 1989) and by DNA sequences that induce bends (Marini et al., 1982; Wu & Crothers, 1984; Diekmann & Wang, 1985; Hagerman, 1985; Koo et al., 1986). Ligation of 21-mer duplexes yielded a series of polymers of length $(dX_{21})_N$ (Figure 1). Assuming 10.5 base pairs per turn (Wang, 1979; Rhodes & Klug, 1981), base substitutions were in phase, 21 base pairs apart. Thus, asymmetric effects of the substitutions on helix structure would be additive with increasing polymer lengths (Ulanovsky et al., 1986). If these substitutions promote asymmetric distortion of the helix, we can expect a decrease in electrophoretic mobility of the helix on polyacrylamide gels (Marini et al., 1982; Wu & Crothers, 1984; Diekmann & Wang, 1985; Hagerman, 1985; Koo et al., 1986; Husain et al., 1988; Hsieh & Griffith, 1989) that is greater than that caused by localized frictional effects on DNA electrophoretic mobility discernible by regularly alternating the lesion from side to side along the DNA. Differences in relative electrophoretic mobilities of the base-substituted multimers increased linearly as polymer size increased (Figures 2 and 3).

Ligation of 16-mers placed base substitutions 16 base pairs apart and alternated the lesions from one side of the helix to the other (Figure 1); this alteration of possible asymmetric perturbation regularly from side to side on the helix will average differences over the length of the helix to give straight DNA (Koo & Crothers, 1988). Decreases in relative electrophoretic mobility in this situation should measure frictional effects caused by only localized distortions. The electrophoretic mobilities of ligated 16-mer duplexes containing G·C, A·C, or O^6-meG·C base pairs alternately on opposite sides of the helix were examined. All the substitutions showed a decrease in mobility on polyacrylamide gel electrophoresis relative to the ligated 16-mers containing G·C (Figure 4 and Table I). However, the ligated 16-mers all showed a smaller decrease in relative mobility per base pair than the ligated 21-mers, even though the density of base substitution per polymer was higher.

Quantitation of changes in relative mobility per base pair caused by base-pair substitution at the two adjacent G·C base pairs (sites 1 and 2) is shown in Table I. All base-pair substitutions, except for G·T at site 1, decreased the mobilities of the ligated polymers relative to those of control polymers; the A·C mismatch caused the greatest overall decrease in mobility, whereas G·T influenced mobilities the least. Moving substitutions between the DNA positions (sites 1 and 2) significantly affected relative gel electrophoretic mobilities (Table I).

Effects of Substitutions on T_m. The melting curves for duplexes containing the above substitutions are shown in Figure 5, and the T_m values determined from these curves are shown in Table I. All substitutions for G·C at either site resulted in a decreased T_m. Substitutions at site 1 gave T_ms of about 66 °C except for G·T, which was less destabilizing. O^6-meG substitutions were more destabilizing at site than at site 2. A·C

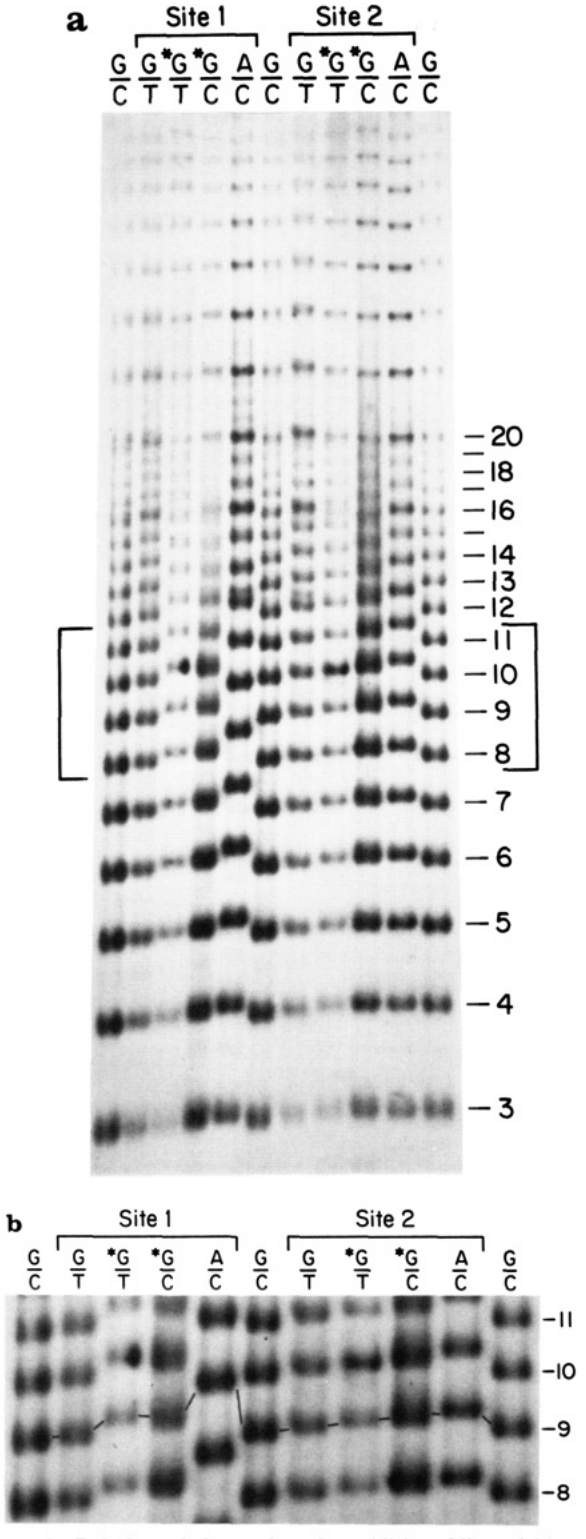

FIGURE 2: Relative gel electrophoretic mobilties of ligated 21-mer duplexes on 4% nondenaturing polyacrylamide. (a) Radiolabeled 21-mer duplexes containing G·T, O^6-meG·T, O^6-meG·C, and A·C base pairs at sites 1 or 2 were ligated and the products electrophoresed as described under Materials and Methods. Control duplexes (G·C base pairs at both sites 1 and 2) are also shown. The sizes of the ligated products (numbers of 21-mer monomers) are indicated along the right side of the autoradiogram. (b) The area enclosed by brackets is shown enlarged. Bands corresponding to polymers composed of nine 21-mer monomers are indicated. *G, O^6-meG.

was more destabilizing than G·T at either site.

DISCUSSION

A mismatch or a base pair containing O^6-meG in DNA is considered a localized defect in the helical structure; in principle, the nature and magnitude of this defect can be influenced by the surrounding sequence. To investigate the effect of DNA sequence on the defect, we have determined T_m values and relative polyacrylamide gel electrophoretic mobilities of duplexes containing G·T, O^6-meG·T, O^6-meG·C, or A·C base pairs in place of G·C at two adjacent positions (sites 1 and 2 defined in Figure 1).

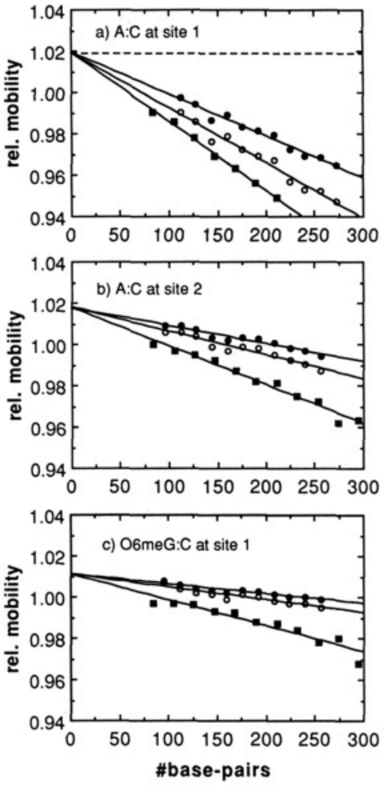

FIGURE 3: Change in relative gel electrophoretic mobility as a function of polymer length. The relative mobilities of base-substituted polymers were calculated from the polyacrylamide gels shown in Figures 2 and 4 by dividing the distance of migration of the substituted polymer by the distance of migration of the control polymer (containing G·C at sites 1 and 2) for polymers of varying lengths. (●) Values for ligated 16-mers corrected to reflect the lower content of lesions in 21-mers relative to 16-mers; (○) ligated 16-mers; (■) ligated 21-mers. (a) Polymers containing A·C at site 1; (b) polymers containing A·C at site 2; (c) polymers containing O^6-meG·C at site 1.

Alteration of Helix Structure by G·T, O^6-meG·T, O^6-meG·C, and A·C Base Pairs. Gel electrophoresis has proven to be useful for detecting helical distortion induced by modified bases or specific sequences (Koo et al., 1986; Husain et al., 1988; Rice et al., 1988; Koo & Crothers, 1988). Base-pair substitutions for G·C decreased the relative electrophoretic mobility of DNA linearly with increasing numbers of ligated monomers. The rank order of apparent base-pair "fit", where fit refers to lack of distortion, at site 1 was G·C = G·T $\gg$ O^6-meG·C > O^6-meG·T $\gg$ A·C and at site 2 was G·C > G·T = O^6-meG·T > O^6-meG·C $\gg$ A·C. A·C caused the largest decrease in electrophoretic mobility and G·T the least relative to G·C at both sites. This results is consistent with the instability of A·C relative to G·T (Aboul-ela et al., 1985; Werntges et al., 1986; Gaffney & Jones, 1989) and also with NMR studies, which demonstrate than an A·C mismatch destabilizes several base pairs on either side of the mismatch (Patel et al., 1984).

A possible alternative to greater instability as an explanation for the larger effect on DNA electrophoretic mobility by A·C relative to that by G·T is that the A·C wobble mispair may

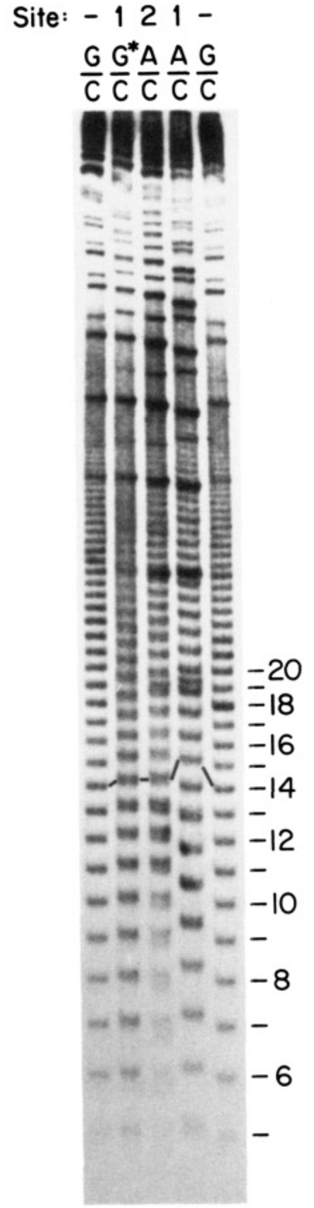

FIGURE 4: Relative gel electrophoretic mobilities of ligated 16-mer duplexes on 4% nondenaturing polyacrylamide. Radiolabeled 16-mer duplexes containing O^6-meG·C or A·C base pairs were ligated and the products electrophoresed as described under Materials and Methods. The sizes of the linear ligated polymers (numbers of 16-mer monomers) are indicated along the right-hand side of the autoradiogram. The bands corresponding to polymers composed of 14 16-mer monomers are indicated. G*, O^6-meG.

be protonated [Topal & Fresco, 1976; Hunter et al., 1986; Sowers et al., 1986; but see Kalnick et al. (1988a)]. Protonation would reduce the effective net charge per unit length of A·C-containing DNA relative to that of control DNA and thus its mobility during gel electrophoresis.

Although gel electrophoresis offers no direct information about the structure of the mismatches, it does allow insights from a comparison of the effects on DNA electrophoretic mobility of the different substitutions. Substitutions at site 1 caused a greater decrease in electrophoretic mobility of the DNA than the same substitutions at site 2 for three of the four base substitutions studied; for the fourth, G·T, the reverse occurred. One possible explanation for this effect of position on the lesion, as detected by mobility differences, may be the base-stacking interactions of the base-pair substitution with its nearest neighbors: T·A and G·C at site 1 and G·C and A·T at site 2. For example, the A·C mismatch should be more stable at site 2 than at site 1, since the only sequence change,

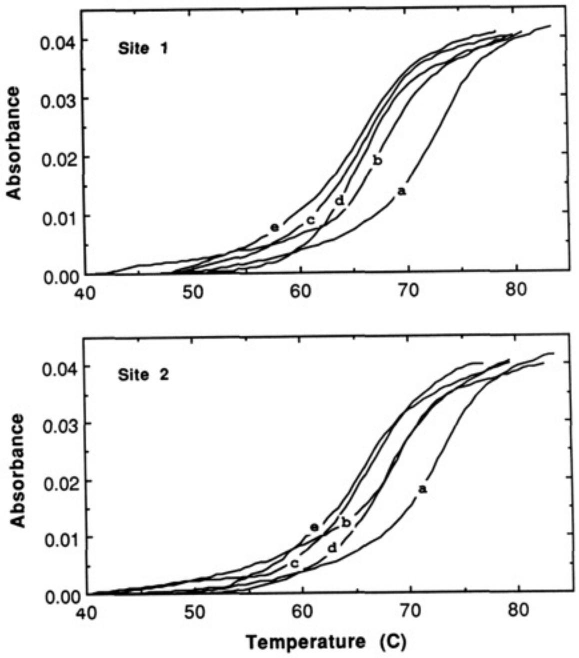

FIGURE 5: Melting curves of 21-mer duplexes shown in Figure 1 containing base substitutions at sites 1 and 2. 3 μg of each complementary strand was annealed and diluted to a volume of 1.2 mL containing 1.4 mM Tris-HCl, pH 7.6, 0.7 mM $MgCl_2$, 0.1 mM EDTA, and 25 mM NaCl. Duplexes were melted by raising the temperature from 40 to 85 °C at a rate of 0.5 °C/min. T_m values were calculated from first-derivative analysis of the melting curves; the values were not corrected for single-strand melting. Base substitutions in the 21-mer duplex correspond to (a) G·C, (b) G·T, (c) O^6-meG·T, (d) O^6-meG·C, and (e) A·C.

1 2
-TAGA- vs -TGAA-
-ACCT- -ACCT-

an A in place of a G in the upper strand, occurs either between T and G (site 1) or between G and A (site 2). The G and A neighbors should be more effective at stabilizing a mismatched base in an intrahelical stacked conformation; work of Tinoco, Ts'o, and others have clearly documented the effects of nearest-neighbor interactions on base-pair stability [reviewed by Saenger (1984)]. In general, purine interactions are the most stabilizing. These same considerations can explain the greater distortion by O^6-meG·C at site 1 since less stacking at site 1 means more time spent melted out-of-helix as a localized bubble.

Substituting G·T for G·C at site 1 or site 2 results in a one-base sequence change in the lower strand:

1 2
-TGGA- vs -TGGA-
-ATCT- -ACTT-

Nearest-neighbor arguments (C and A at site 1 and T and C at site 2) predict that this substitution at site 2 is less stable, and therefore can explain the one case found (Table I) wherein substitution at site 2 causes a greater decrease in mobility than substitution at site 1.

Substitution of O^6-meG·T changes the sequence in both strands, making it difficult to predict which stacking interactions will predominate. The experimental results, however, indicate that the purine interactions in the O^6-meG-containing strand predominate.

This stabilizing effect of purine nearest neighbors may partly explain the results of Hseih and Griffith (1989); they were unable to detect a change in the mobility of ligated 32-mers containing mismatches located between G·C and A·T neighbors. In addition, detection of mobility differences may have been more difficult in their study since the number of mismatches relative to normal base pairs was lower (1 A·C per 32-base oligomer compared to 1 A·C per 21-base oligomer in our study).

Asymmetry of Helix Defects Caused by Mismatched Base Pairs. The effect of O^6-meG·C and A·C on the relative electrophoretic mobilities per base pair was significantly greater for ligation of 21-mers, which placed the lesions regularly spaced on the same face of the helix, compared to that for ligation of 16-mers, which placed the lesions regularly spaced and alternating from one face to the other (Figure 1). Since ligated 16-mers contain a greater density of lesions, they might be expected to show the largest decreases in DNA electrophoretic mobility. The fact that the ligation of 21-mers showed the larger decreases suggests that mismatches and O^6-meG-containing base pairs, at least A·C and O^6-meG·C, contain an asymmetric component. The placement of the asymmetric mismatches so that they are oriented in the same direction relative to each other on the helix enables detection of their effect on the electrophoretic mobility of the helix on polyacrylamide gels. In the 16-mers, opposing the asymmetries by alternating the mismatch from side to side on the helix "factors out" the asymmetric effect on electrophoretic mobility and leaves the frictional drag component, which is probably due to the equilibrium of the mismatch between intrahelical and looped-out configurations.

The basis for the structural asymmetry may lie with the different stacking interactions present in the sequence on the two complementary strands. For the A·C mismatch at site 2, it is possible that the A spends more time stacked in helix than its opposing C because of greater stabilizing nearest-neighbor forces. If this model is correct, we would expect the amount of asymmetry, and thus the global effect on the DNA molecule, to be proportional to the relative time spent by the two bases of the mismatch in an extrahelical disposition; indeed, a fixed one-base loop (Fresco & Alberts, 1960), one extreme of the model, dramatically affects the gel electrophoretic mobility of DNA, indicating significant distortion of the helix (Hsieh & Griffith, 1989).

Effects of G·T, O^6-meG·T, O^6-meG·C, and A·C on Duplex Stability. The results show that base substitution or modification at either site 1 or site 2 decreased the T_m of the oligonucleotide duplex. The stability of a duplex containing a modified or mismatched base pair, as measured by T_m, should reflect the stability of the altered base pair (Aboul-ela et al., 1985; Werntges et al., 1986; Gaffney & Jones, 1989). Overall, the rank order base-pair stability at site 1 was G·C > G·T > O^6-meG·T = O^6-meG·C = A·C and at site 2 was G·C > G·T = O^6-meG·C > O^6-meG·T > A·C.

Our results support the ranking G·C > G·T > O^6-meG·C > O^6-meG·T > A·C reported by others (Aboul-ela et al., 1985; Gaffney & Jones, 1989) from measurements for these same defects at a single site with A and T nearest neighbors. In adddition, our results extend the study to another site with different nearest neighbors, G and A, and show that shifting the lesions by a single base-pair can change relative DNA stability. Greater stability was achieved by moving O^6-meG from site 1 to site 2; this is consistent with the greater purine nearest-neighbor interactions at site 2.

In contrast, Pauly et al. (1988), using 16-mer duplexes of H-*ras* sequence (nucleotides 25–40), found no significant effects on either T_m values or circular dichroism spectra due to moving O^6-meG·C from site 1 to site 2 in an equivalent sequence. The different conditions used by Pauly et al., 1.0 M NaCl compared to the 0.7 mM $MgCl_2$ and 25 mM NaCl used here, may explain their inability to detect small positional effects on stability since high salt stabilizes the helix by charge neutralization and may mask small effects from a localized defect.

We detected no changes in T_m concomitant with moving either G·T or A·C from site 1 to site 2. Whereas, significant differences in electrophoretic mobilities per base pair were caused by these same moves.

Conclusions. These studies indicate, for the first time, that O^6-meG and single-base mismatches cause global effects on the DNA molecule by their ability to cause a small but measurable asymmetric distortion or bend at the site of damage; the effect of base substitution on DNA electrophoretic mobility was greatest when the substitutions were all located along one face of the helix. Such effects on the trajectory of the DNA molecule may be a factor in how enzymes recognize such lesions in the DNA helix.

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Structural Studies of Bean Pod Mottle Virus, Capsid, and RNA in Crystal and Solution States by Laser Raman Spectroscopy[†]

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ABSTRACT: Structures of protein and RNA components of bean pod mottle virus (BPMV) have been investigated by use of laser Raman spectroscopy. Raman spectra were collected from both aqueous solutions and single crystals of BPMV capsids (top component) and virions (middle and bottom components, which package, respectively, small and large RNA molecules). Analysis of the data permits the assignment of conformation-sensitive Raman bands to viral protein and RNA constituents and observation of structural similarities and differences between solution and crystalline states of BPMV components. The Raman results show that the protein subunits of the empty capsid contain between 45% and 55% β-strand and β-turn secondary structure, in agreement with the recently determined X-ray crystal structure, and that this total β-strand content undergoes a small increase (~5%) with packaging of RNA. The subunits are relatively deficient in α-helix secondary structure, estimated at <25%, and therefore must contain extensive amounts (>20%) of loops and irregular chain conformations. The Raman spectra also show the following: (1) The molecular environments of as many as four tryptophan residues per subunit are altered upon packaging RNA, resulting in stronger 1N–H hydrogen bonding for two Trp residues and more hydrophobic environments for two indole rings. (2) Hydrogen-bonding states of the seven Tyr residues per subunit do not change detectably when RNA is packaged. At least five tyrosine OH groups are involved exclusively as strong hydrogen bond donors to protein acceptor groups, which suggests restricted access of solvent H_2O molecules to these parahydroxyls. (3) Hydrogen-bonding states of the 14 Cys S–H groups are the same in top, middle, and bottom components. (4) Bands of the ionized carboxyl groups of Asp and Glu are detected in all viral components and no differences occur in these bands with RNA packaging. (5) Significant rearrangement of aliphatic side chains within the viral capsid may occur with packaging of RNA. (6) BPMV middle and bottom components exhibit no detectable differences in subunit secondary structure. A comparison of Raman spectra of crystal and solution states of the BPMV middle component reveals only minor structural differences between the two, and these are restricted almost exclusively to Raman bands of RNA in the region of assigned phosphodiester conformation markers. Although in both the crystal and solution only C3′ endo/anti nucleosides are detected, the crystal exhibits a weaker 813-cm^{-1} band and stronger 870-cm^{-1} band, which suggests that ~8% (±3% uncertainty) of the nucleotides have O–P–O torsions configured differently in the crystal from that in the solution.

Bean pod mottle virus (BPMV) is a member of the comovirus family. Its virion is characterized by a divided genome and a capsid subunit containing two different polypeptide chains (heterodimer). The two single-stranded RNA molecules are packaged separately in capsids that are composed of identical numbers of heterodimers. Isolation of BPMV from infected plants results in a mixture of the two ribonucleoproteins, as well as empty protein shells. Separation by sucrose gradient sedimentation yields a top component of 54 S [5.0

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