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7-Methylguanine in poly(dG-dC)·poly(dG-dC) facilitates Z-DNA formation

(methylation/mutagenesis/circular dichroism/left-handed DNA)

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ABSTRACT **Poly(dG-dC)·poly(dG-dC) was methylated at the N-7 position of guanine through the use of dimethyl sulfate. The conversion of poly(dG-dC)·poly(dG-dC) from B-DNA to the Z-DNA form was followed by measuring both the circular dichroic spectra and changes in the absorbance ratio A_{295}/A_{260}. Increasing methylation steadily decreases the amount of NaCl or $MgCl_2$ that is required to convert the polymer from B-DNA to Z-DNA. At 100% methylation of the guanine residues, the modified polymer is fully converted to Z-DNA in a physiological salt solution. Kinetic experiments show that methylation markedly accelerates the speed of the conversion from B-DNA to Z-DNA in the presence of added salt. These effects may be partly due to the positive charge on guanine that accompanies N-7 methylation.**

DNA usually is found as a right-handed double helix (B-DNA) in which all purine and pyrimidine residues are in the *anti* conformation, and the molecule has a central core of Watson–Crick base pairs. Recent crystallographic analyses have demonstrated that it is possible to form a left-handed conformer of DNA in which the backbone has an irregular or zigzag array (Z-DNA) (1–4). This form is most readily seen in a sequence of DNA with alternating guanine and cytosine residues but is also found with alternating purine and pyrimidine residues (5). In left-handed Z-DNA, the Watson–Crick base pairs are found on the outside of the molecule, and the guanine residues are in the *syn* conformation. The molecule has only one deep groove instead of the two grooves in the right-handed B-DNA form. Chemical modifications of DNA can change its stability, and we are interested in the conversion of B-DNA to Z-DNA. Alkylating agents such as alkyl sulfates, *N*-nitroso compounds, and diazoalkanes react with DNA and polynucleotides to form a variety of products (6–8). Many of these are mutagens, and some are highly carcinogenic. Dimethyl sulfate reacts with DNA, and the main product is 7-methylguanine.

Pohl and Jovin (9) discovered that raising the salt concentration of a poly(dG-dC)·poly(dG-dC) solution produced a cooperative inversion of the circular dichroic spectra of this polymer. We now interpret the low-salt form as B-DNA and the high-salt form as Z-DNA because it has been shown in a laser Raman study that the high-salt form is the same as Z-DNA in crystals (10). The carcinogen acetylaminofluorene will convert right-handed poly(dG-dC)·poly(dG-dC) into Z-DNA when the carcinogen has reacted with approximately 20% of the guanine residues (11–13). This bulky group cannot attach to the C-8 position of the guanine residue in B-DNA but can do so readily in left-handed Z-DNA because of the *syn* conformation.

We have been interested in examining the effect of methylation at the N-7 position of guanine and its influence on the B- to Z-DNA transition by using poly(dG-dC)·poly(dG-dC) as substrate. We have found that this methylation facilitates the formation of Z-DNA. When the guanine residues are fully methylated, the molecule exists predominantly as left-handed Z-DNA in physiological salt solutions. The introduction of methyl groups reduces the salt concentration required for the conversion of B-DNA to Z-DNA, and it occurs more rapidly. These experiments raise the possibility that methylation of guanine residues in Z-DNA may serve as a focus for altering the local conformation of DNA.

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MATERIALS AND METHODS

Chemicals. Poly(dG-dC)·poly(dG-dC) was purchased from P-L Biochemicals, dimethyl sulfate was from Aldrich, and tributylamine was from Eastman. Instruments used were a Cary 60 for circular dichroism measurements and a Zeiss PMQ 2 and Perkin–Elmer model 330 for spectroscopic measurements. Waters Associates high-pressure liquid chromatograph was used with a μ-Bondapak C18 Waters Associates column.

Polymer Methylation. Tributylamine (300 μl) and dimethyl sulfate (100 μl) were added to 5 ml of 0.5 M NaCl containing 100 A_{260} units of poly(dG-dC)·poly(dG-dC). The basic solution was agitated at 4°C, and aliquots were taken out at various times for analysis. Further additions of tributylamine and dimethyl sulfate at 24 and 48 hr were in quantities consistent with those described by Pochon and Michelson (14). Throughout the reaction, the pH was maintained between 7.8 and 8.8. The solution was then dialyzed exhaustively against 10 mM NaCl/15 mM Tris·HCl, pH 7.2.

To analyze the extent of methylation, the DNA was precipitated with 95% ethanol and treated with 5 μl of perchloric acid (72%) at 100°C for 1 hr to remove the bases. The solution was then neutralized with triethanolamine, and insoluble products were removed by centrifugation. The bases were separated by passage through a Waters Associates μ-Bondapak C18 column used with a Waters Associates liquid chromatograph (model U6K injector, model 152 Altex absorbance detector at 254 nm, model 6000A pump), with 0.01 M $NH_4H_2PO_4$ containing 0–20% methanol as solvent at 2 ml/min. The bases were eluted in the sequence cytosine, guanine, 7-methylguanine. The mole fraction of each base was calculated by the height-times-width-at-half-height method, in which the approximate peak area is determined by the formula: (peak height × peak width at half height)/2. The guanine was 25% methylated after 3 hr of incubation and 50% at 10 hr. After 1 day of incubation, 75% of the guanine bases were methylated. By 2.5 days of incubation, virtually 100% of the guanine bases were modified. The analysis revealed that 7-methylguanine was the sole product for the first 50 hr of incubation. After that, small amounts of methylated cytosine derivatives appeared. When 100% of the guanine residues were eluted as 7-methylguanine, only 4% of the cytosine residues had been methylated.

RESULTS

Methylation of deoxyguanosine at the N-7 position of the guanine residue modified the ultraviolet absorption spectrum. The λ_{max} for deoxyguanosine was 254 nm, whereas that for 7-methyldeoxyguanosine was 257 nm. Further, there was a considerable loss of absorbing power. The extinction coefficient (ε_{max}) at neutral pH was 13.0×10^3 for deoxyguanosine and only 8.5×10^3 for 7-methyldeoxyguanosine (ε_{258}) (15). These changes are reflected in the UV spectra of the polymers shown in Fig. 1. The ultraviolet absorption peak of methylated poly(dG-dC)·poly(dG-dC) markedly decreased with a slight shift to longer wavelengths. This shift is associated with increased absorption at longer wavelengths, especially in the region greater than 300 nm.

The conversion of right-handed B-DNA to left-handed Z-DNA in high-salt solutions is associated with a near-inversion of the circular dichroic spectrum (9). Fig. 2 shows the circular dichroic spectra of poly(dG-dC)·poly(dG-dC) containing 0%, 52%, and 90% 7-methylguanine residues. There was a steady modification of the low-salt spectra associated with methylation. The positive band near 270 nm was completely replaced by a negative band at 90% modification. A new positive band around 240 nm was formed with increasing methylation. Methylation of the guanine residues was associated with both a loss of absorption (Fig. 1), and a loss in the ellipticity (Fig. 2). Despite the decreased ellipticity, raising the salt concentration was still

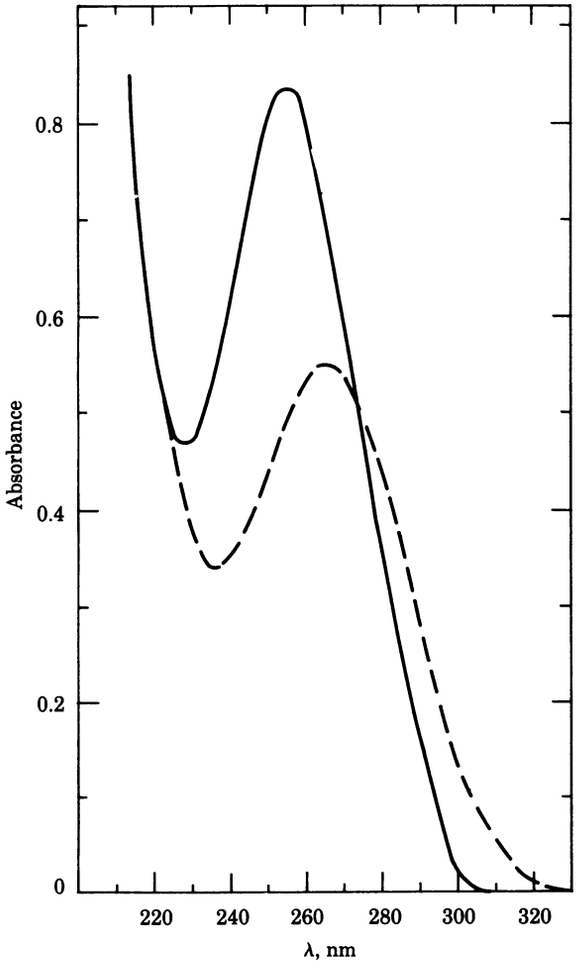

FIG. 1. Absorbance spectra of poly(dG-dC)·poly(dG-dC) (——) and its derivative with 100% of the guanine residues methylated (----) in 0.01 M NaCl/15 mM Tris·HCl, pH 7.2.

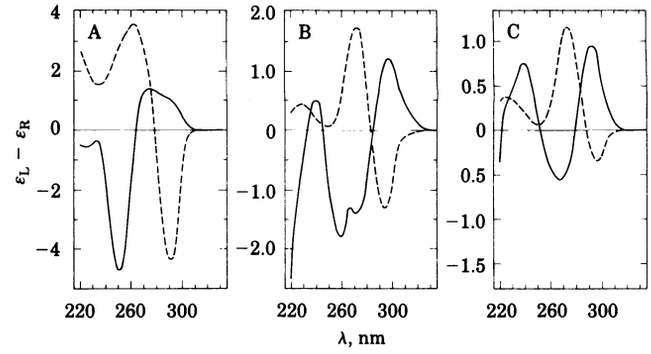

FIG. 2. Circular dichroic spectra of unmodified and modified poly(dG-dC)·poly(dG-dC) in 0.01 M NaCl (——) and 3.0M NaCl (----). Percentage of 7-methylguanine residues: 0% (*A*), 52% (*B*), and 90% (*C*).

associated with a near-inversion of the spectra in both the 52%- and 90%-modified polymers. This is consistent with a conversion to the left-handed Z-DNA form of the molecule when salt is added, even though the molecule is now methylated at position N-7 of the guanine residue.

Pohl and Jovin have shown that the conversion of poly(dG-dC)·poly(dG-dC) from one form to the other is associated with a sharp increase in the ratio A_{295}/A_{260} (9). Similar changes occurred in methylated poly(dG-dC)·poly(dG-dC). Fig. 3 shows these effects for the addition of NaCl (Fig. 3*A*) and $MgCl_2$ (Fig.

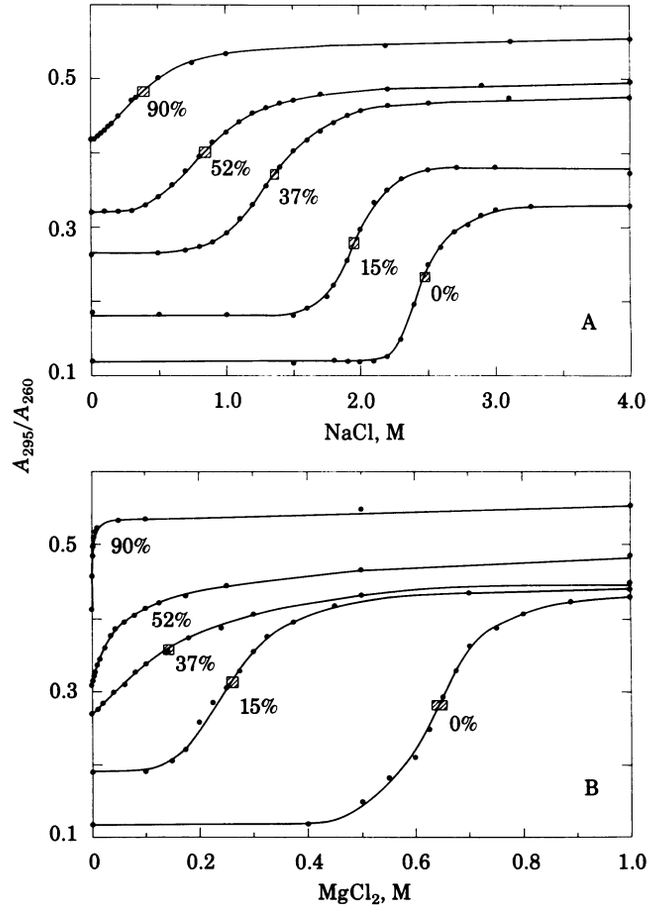

FIG. 3. Changes in the A_{295}/A_{260} ratio as a function of the molarity of NaCl (*A*) and $MgCl_2$ (*B*) for different polymers. The percentage of modified guanine is shown. ⧈, Midpoint of the rise in the absorbance ratio.

3*B*) as a function of varying degrees of methylation of the guanine residue. The ratio of A_{295}/A_{260} was elevated as the amount of methylation increased, even before salt addition. Nonetheless, addition of salt was associated with a sharp rise in this ratio at a concentration that was characteristic of the degree of methylation. In the absence of methylation, the midpoint for the conversion from B- to Z-DNA was near 2.5 M NaCl. Methylation had the effect of steadily decreasing the amount of NaCl required. The midpoint for 90% methylation of the guanine residues in poly(dG-dC)·poly(dG-dC) was close to 0.35 M NaCl. The conversion from one form to the other was sigmoidal, except for 90% methylation.

The magnesium ion is much more effective than sodium for the conversion of poly(dG-dC)·poly(dG-dC) from one form to the other (9). The same was true for the methylated polymer (Fig. 3*B*). The midpoint for conversion was approximately 640 mM for the unmethylated polymer, 250 mM for the 15% methylated polymer, and near 130 mM for the 37% methylated polymer. At 52% methylation, the midpoint was more difficult to assign but may be near 50 mM. By 90% methylation, the polymer appeared to have been largely converted to Z-DNA at 10 mM magnesium.

The effect of small amounts of magnesium led us to investigate the circular dichroism of poly(dG-dC)·poly(dG-dC) with various degrees of methylation in a "physiological" salt solution—that is, one containing 150 mM NaCl and an effective concentration of 7 mM $MgCl_2$. The circular dichroic curves for various degrees of methylation are shown in Fig. 4. At 37% methylation of the guanine residues, the curve was similar to that of right-handed B-DNA, with the spectrum modified slightly by the presence of the methyl group. As the percentage of methylation increased, the strong negative peak at 250 nm decreased steadily. The strong positive peak at 290 nm decreased and finally assumed a negative position at 100% methylation of the guanine residue. The 100% methylated polymer was fully converted to Z-DNA in that further addition of NaCl or $MgCl_2$ had no effect. Thus, in a physiological salt solution, the fully methylated polymer is in the left-handed conformation as shown by the circular dichroic measurements and as suggested by changes in the absorbance ratios (Fig. 3).

The conversion from a B-type spectrum to a Z-type spectrum was associated with a shift in the positive band from 290 nm to 275 nm. An interesting effect was seen in comparing the position of these two bands when sodium and magnesium were used to effect the conversion. At 75% methylation of the polymer, a positive peak was seen at 290 nm in 150 mM NaCl/7.0 mM $MgCl_2$. However, in a spectrum with 7.0 mM $MgCl_2$, alone, the positive peak was no longer found at 290 nm but rather had a maximum at 277 nm in a position similar to that for the 90% methylation curve. This suggests that the addition of sodium ions diluted the effectiveness of the magnesium ions in facilitating the conversion from B- to Z-DNA.

Pohl and Jovin (9) have looked at the kinetics of the conversion brought about by the addition of salt. They showed that the conversion was highly cooperative and that the first-order kinetics was fairly slow—in a time range of 100–1000 sec. We have asked whether methylation of guanine residues, even at fairly low levels, would influence the kinetics of the conversion. The conversion from B- to Z-DNA was monitored by measuring ellipticity as a function of time at 290 nm. The zero-time measurement was made in 10 mM NaCl, and the solution was then shifted in salt concentration to 3.2 M NaCl. The kinetics of the conversion were quite different for the methylated compared to the unmethylated material (Fig. 5). The conversion occurred more rapidly with methylation. The speed of the conversion could be expressed by measuring the relaxation time, τ. The relaxation time for unmodified poly(dG-dC)·poly(dG-dC) was 580 sec. The relaxation time decreased considerably upon methylation. The relaxation times for 15%, 37%, and 52% methylation decreased approximately in a linear plot, which extrapolated back to 280 sec at 0% modification, compared to 580 sec which actually was observed for 0% modification. This suggests that even small numbers of guanine residues with methyl groups substituted at the N-7 position could act as a focus for facilitating the conversion of B- to Z-DNA. The conversion of B- to Z-DNA in the methylated polymer appears to be a process

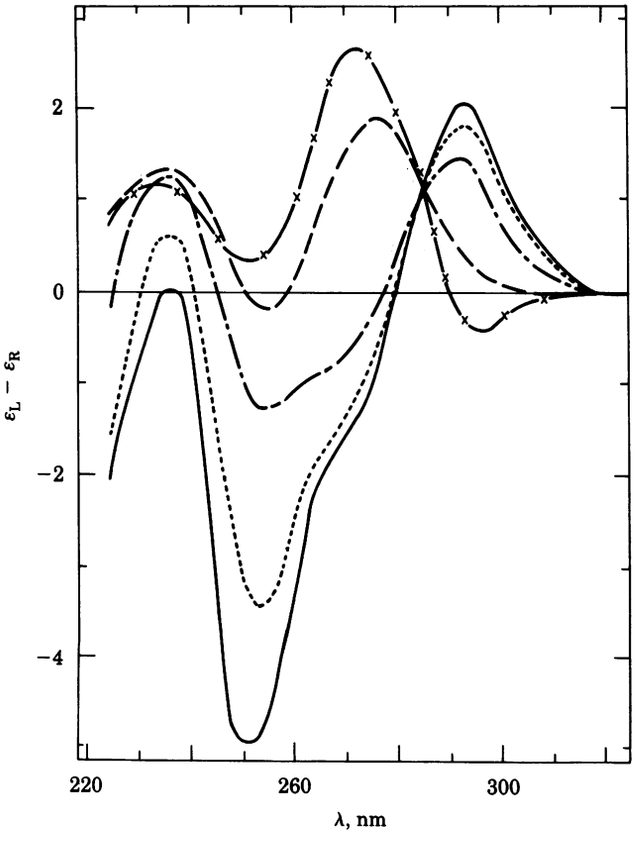

FIG. 4. Circular dichroic spectra of poly(dG-dC)·poly(dG-dC) containing different percentages of 7-methylguanine residues in 150 mM NaCl/7.5 mM $MgCl_2$/0.5 mM EDTA/10 mM Tris·HCl, pH 7.2. x——x, 100%; — — —, 90%; — · —, 75%; ----, 52%; ——, 37%.

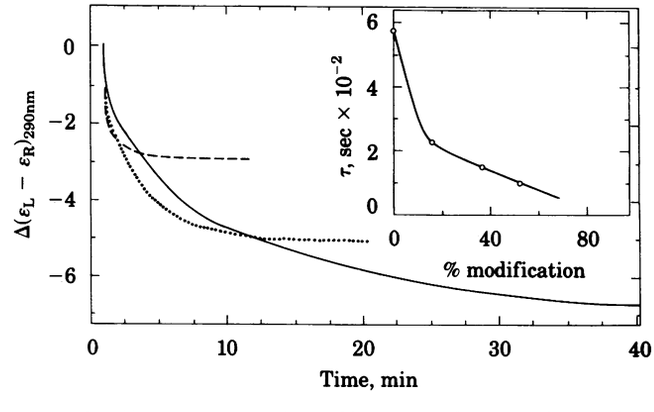

FIG. 5. Change in ellipticity at 290 nm as a function of time after a shift in salt concentration from 0.01 M to 3.2 M NaCl at 28°C. ——, Poly(dG-dC)·poly(dG-dC); ..., 15% methylated polymer; — — —, 37% methylated polymer. (*Inset*) Relaxation time as a function of methylation of the guanine residue at the N-7 position.

that differs in a fundamental fashion from the conversion that occurs in the absence of methylation.

DISCUSSION

7-Methylguanine is lost from DNA by chemical hydrolysis at a significant rate at neutral pH (8, 16, 17). The breakdown is associated with the formation of apurinic sites. Accordingly, care was taken with the solutions to minimize this effect. In this investigation, the methylated polymers were stored at −20°C, and aliquots of this preparation were used to make only one measurement. This is important because evidence for the instability could be detected when multiple measurements were made with the same sample.

In degrading the samples to analyze the extent of modification, perchloric acid was used to produce the bases. The usual technique for obtaining nucleotides involves the use of an enzyme such as micrococcal nuclease. However, this could only be used for those samples that had low degrees of methylation. Once the degree of methylation was 75% or greater, the polymers became resistant to the nuclease digestion in 10 mM $CaCl_2$. This was interpreted as being associated with the formation of nuclease-resistant Z conformation.

Fig. 3 shows that the methylation of guanine residues in poly(dG-dC)·poly(dG-dC) produces a steady and consistent drop in the concentration of salt that is needed to convert from the B to the Z conformation, as judged by the change in the A_{295}/A_{260} ratios. It is quite likely that the major effect produced by salt in the conversion of B- to Z-DNA is associated with a screening by the positively charged cations of the negatively charged phosphate groups. The phosphates on the two strands are closer together in Z-DNA than in the B conformation (1). Less salt is required as the methylation proceeds. One effect of methylation is associated with the introduction of a positive charge in the guanine ring. We suggest that this produces much the same effect as increasing the salt concentration, which clamps down on the ionic atmosphere of cations surrounding negatively charged phosphates. In essence, fewer cations are needed because the positively charged guanine residues provide neutralization for their neighboring phosphate groups.

The kinetic experiments in Fig. 5 were designed to measure the effects of varying amounts of methylation on the ease with which the conversion of B- to Z-DNA occurs. In our studies, we could see that a few methyl groups have profound effects as methylation lowers the relaxation time for the conversion in a discontinuous fashion.

One of the major differences between Z-DNA and B-DNA is related to the distance between phosphate groups on opposite strands, which are closer to each other across the deep groove in Z-DNA than they are in B-DNA. At low ionic strength, B-DNA is the stable form of poly(dG-dC)·poly(dG-dC). Part of its stability is derived from the fact that in this conformation, interstrand phosphate repulsion is minimized. In converting from B- to Z-DNA, the two strands must come together. In effect, this represents an activation energy that limits the rate at which the process occurs. It is reasonable to believe that there is a lowering of this activation energy associated with methylation. Because the positive charge on the 7-methylguanine residue neutralizes adjoining phosphate groups, there is a consequent lowering of the repulsive energy between phosphate groups on opposite strands. It is likely that the locus of this charge is the point at which the conversion occurs, and the rate of the conversion is considerably faster because of the lowering of the interstrand phosphate repulsion energy associated with charge neutralization. An explanation of this type suggests that the presence of only a few methyl groups on poly(dG-dC)·poly(dG-dC) would result in a significant lowering of the relaxation time.

An extended study by Ramstein *et al.* (16) has shown that methylation of DNA produces an increased flexibility of the molecule, a loss of viscosity, and a lowering of the melting temperature. All of these effects may be related to the charge neutralization associated with methylation. It is likely that charge neutralization cannot account for all of the effects. Methylation undoubtedly modifies the hydration around the molecule, and this, in turn, will result in altered properties. However, the changes in the properties of DNA are qualitatively in agreement with what would be anticipated if the major component were due to charge neutralization.

It is interesting to compare the effects of methylation of guanine residues at the N-7 position with methylation of cytosine residues at the C-5 position in poly(dG-dC)·poly(dG-dC). Behe and Felsenfeld (18) have made the interesting observation that cytosine residue C-5 methylation has a profound effect in facilitating the B to Z transition in this polymer, somewhat greater than that which we observe for guanine residue N-7 methylation. At first this seems surprising, because there is no change in charge associated with cytosine methylation. We believe the cytosine residue methylation effect is associated with a more stable environment for the C-5 methyl group in Z-DNA. In Z-DNA, the cytosine residue C-5 methyl group is on the outside of the molecule, where it closely abuts two groups, C1′H and C2′H of the adjoining guanosine residue (4). There it forms a stable hydrophobic bond. Together with modifications in the hydration pattern, this may considerably stabilize the Z-conformation.

It has been shown that mitomycin C facilitates the conversion of B-DNA to Z-DNA even more strongly. Poly(dG-dC)·poly(dG-dC) is completely converted to the Z form when only 25% of the residues have reacted (19). However, the chemistry of this reaction is not yet understood. In the case of acetylaminofluorene reacting with the C-8 position of the guanine residue, approximately 20% occupancy converts the polymer to Z-DNA (11–13). There are strong steric reasons that prevent its incorporation into B-DNA, whereas the ready accessibility of the imidazole ring on the outer surface of Z-DNA undoubtedly accounts for its stability.

Methylation of guanine residues in the N-7 position is promoted by many mutagens and carcinogens. Nature has learned how to respond to this damage because a glycosylase for 7-methylguanine has been found in several cell types (20). It is likely that an equilibrium is found between the B and Z conformations of poly(dG-dC)·poly(dG-dC), and the present results suggest that guanine residue N-7 methylation modifies this equilibrium in favor of the Z conformation. It is possible that such an equilibrium is also found in DNA *in vivo*. If that is the case, then the biological effects of N-7 methylation of guanine residue may be associated with a similar alteration of this equilibrium.

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