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Alternative Conformations of DNA Modified by N-2-Acetylaminofluorene

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Modification of DNA by the carcinogen N-acetoxy-N-2-acetylaminofluorene gives two adducts, a major one at the C-8 position of guanine and a minor one at the N-2 position with differing conformations. Binding at the C-8 position results in a large distortion of the DNA helix referred to as the "base displacement model" with the carcinogen inserted into the DNA helix and the guanosine displaced to the outside. The result is increased susceptibility to nuclease S_1 digestion due to the presence of large, single-stranded regions in the modified DNA. In contrast, the N-2 adduct results in much less distortion of the helix and is less susceptible to nuclease S_1 digestion.

A third and predominant adduct is formed in vivo, the deacetylated C-8 guanine adduct. The conformation of this adduct has been investigated using the dimer dApdG as a model for DNA. The attachment of aminofluorene (AF) residues introduced smaller changes in the circular dichroism (CD) spectra of dApdG than binding of acetylaminofluorene (AAF) residues. Similarly, binding of AF residues caused lower upfield shifts for the H-2 and H-8 protons of adenine than the AAF residues. These results suggest that AF residues are less stacked with neighboring bases than AAF and induce less distortion in conformation of the modified regions than AAF.

An alternative conformation of AAF-modified deoxyguanosine has been suggested based on studies of poly(dG–dC)•poly(dG–dC). Modification of this copolymer with AAF to an extent of 28% showed a CD spectrum that had the characteristics of the left-handed Z conformation seen in unmodified poly(dG–dC)•poly(dG–dC) at high ethanol or salt concentrations. Poly(dG)•poly(dC), which does not undergo the B to Z transition at high ethanol concentrations, did not show this type of conformational change with high AAF modification. Differences in conformation were suggested by single-strand specific nuclease S_1 digestion and reactivity with anticytidine antibodies. Highly modified poly(dG–dC)•poly(dG–dC) was almost completely resistant to nuclease S_1 hydrolysis, while, modified DNA and poly(dG)•poly(dC) are highly susceptible to digestion. Two possible conformations for deoxyguanosine modified at the C-8 position by AAF are compared depending on whether its position is in alternating purine–pyrimidine sequences or random sequence DNA.

Key words: acetylaminofluorene, Z-DNA, base displacement model, DNA conformation, circular dichroism (CD), NMR, nuclease S_1 digestion

There is increasing evidence which suggests that covalent binding of carcinogens to cellular macromolecules, particularly nucleic acids, is the initial critical event in the encounter between an environmental carcinogen and target cells in the exposed host [1,2]. Therefore, to understand carcinogenesis at a molecular

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level it becomes necessary to determine the complete chemical structure of the carcinogen-macromolecular adducts, the associated conformational changes in the target macromolecules, and finally to relate these chemical and physical alterations to possible aberrations in the functional properties of the chemically modified macromolecules.

A complicating feature in attempting to relate chemical structure to functional effects is the fact that with most of these agents more than one type of nucleoside adduct in DNA is formed. This is also true with the simpler alkylating agents in which almost every nitrogen and oxygen residue of all the nucleic acid bases can be modified [3]. In the case of N-2-acetylaminofluorene (AAF), Miller et al [4] first demonstrated that both RNA and DNA can react nonenzymatically in vitro with the N-acetoxy derivative of AAF. The major product obtained from hydrolysates of the modified DNA is N-(deoxyguanosin-8-yl)AAF [5], and a minor component 3-(deoxyguanosin-N^2-yl)AAF [6,7] (Fig. 1a,b).

The same two nucleic acid derivatives were found in rat liver DNA when AAF was administered in vivo [6]. However, after in vivo application of N-hydroxy-AAF, about 80% of the C-8 adduct was in the deacetylated (AF) form [8–10] (Fig. 1c). In addition to the guanine adducts, there are studies suggesting that AAF may also modify adenine residues in nucleic acids [11–13], although this adduct has not been actually isolated and characterized. Since modification of the guanosine residues at C-8 position with AAF or AF as well as at N^2 positon with AAF in DNA could be associated with different conformational changes, we have studied the conformations of all three types of adducts.

CONFORMATION OF AAF MODIFIED DNA

Fundamental to an understanding of the functional consequences of nucleic acid modification by bulky carcinogens such as AAF is information on two points: (1) the orientation within a single- or double-stranded nucleic acid of the covalently bound carcinogen residues, and (2) possible alterations in the native conformation of the nucleic acid resulting from this modification. In the case of AAF modification of the C-8 position of guanine residues in double-stranded DNA, considerable evidence has been obtained for a conformational distortion which we termed "base displacement model" [14, 15].

The first feature of this model is that the attachment of the AAF residue to the 8-position of G is associated with a change in glycosidic N^9-$C^{1'}$ conformation

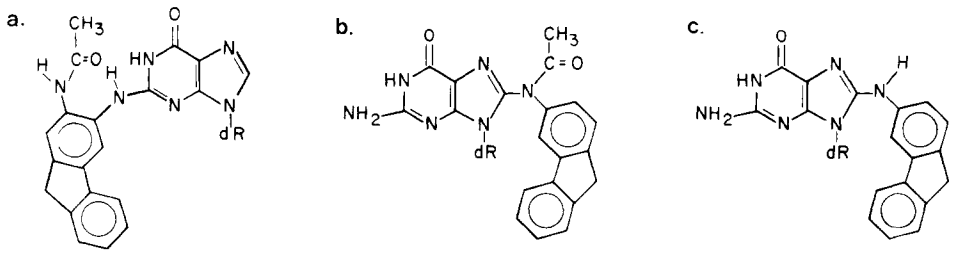

Fig. 1. Structures of DNA adducts: (a) 3-(deoxyguanosine-N^2-yl)-N-acetyl-2-aminofluorene; (b) N-(deoxyguanosin-8-yl)-N-acetyl-2-aminofluorene; (c) N-(deoxyguanosin-8-yl)-2-aminofluorene.

from the *anti* conformation of nucleosides in nucleic acids with Watson-Crick geometry to the *syn* conformation. Evidence for this is restricted to a study of molecular models of AAF-G which indicates severe steric hindrance between AAF and the ribose, or deoxyribose, of the nucleoside, unless the guanine base is rotated on the glycoside bond from the *anti* to *syn* conformation.

The second major feature of this model is that there is a stacking interaction between AAF and a base adjacent to the substituted G residue.

These changes are best illustrated in a computer-generated stereoscopic display of a double-stranded DNA fragment (Fig. 2). The computer display allows one to readily perform rotation around appropriate bond angles while obtaining a three-dimensional image of the molecular structure on a video screen. In the display, the modified base has been rotated around the glycosidic bond from *anti* to the *syn* conformation to avoid the steric hindrance. In addition, the planar fluorene ring system is inserted into the helix occupying the former position of the displaced guanine residue. It is also evident that the G residue displaced by AAF in the double helix cannot base pair with the C residue on the complementary strand, and during the process of replication or transcription no base pairing at this position could occur. We have obtained experimental evidence of the base displacement model from proton magnetic resonance, and CD spectra of modified oligonucleoties [16, 17]. A similar model, called the "insertion-denatur-

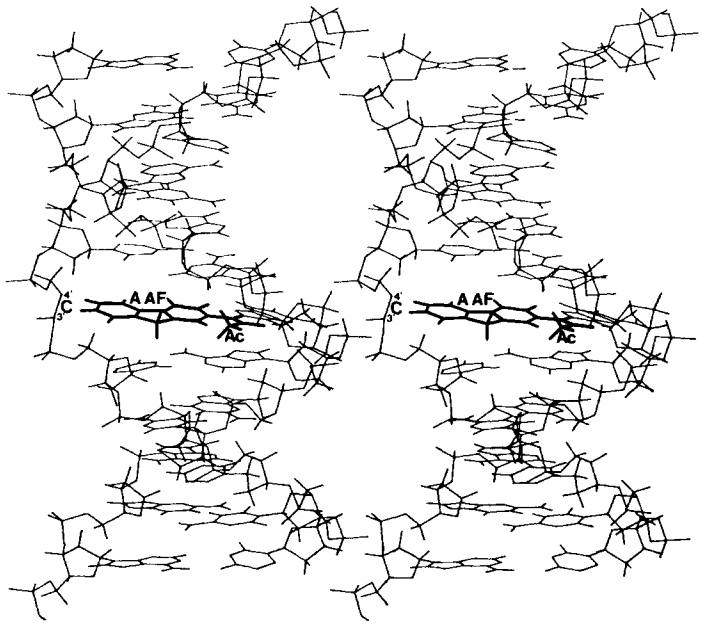

Fig. 2. A stereoscopic view of the "base displacement model" of AAF-DNA adducts. The guanine to which the AAF is attached has been rotated out of the helix and the AAF moiety is inserted into the helix and stacked with the bases above and below. AC designates acetyl group of AAF. The cytosine (marked C) residue on the opposite strand would overlap with the AAF residue; therefore, it has been removed and the 3′ and 4′ carbon atoms of the corresponding deoxyribose in the DNA backbone are indicated. In reality, this C probably rotates out from the helix to accommodate the AAF; the exact conformation is not known, although there is evidence that this region of DNA is "single stranded."

ation model" has been proposed by Fuchs and Daune [18, 19]. They modified native calf thymus DNA with N-acetoxy-AAF, or with the 7-fluoro (AAFF) and 7-iodo (AAIF) derivatives of N-acetoxy-AAF. The technique of electric dichroism was then used to determine the orientation of the covalently bound fluorene ring to the long axis of the DNA. The results clearly indicated that in the case of DNA–AAF and DNA–AAFF, the fluorene ring lies almost perpendicular to the helix axis, the angle being 80°. On the other hand, with the iodo derivative, DNA–AAIF, they found the angle between the transition moment of the fluorene molecule and the helix axis to be only 60°. It seems likely, therefore, that in the case of DNA–AAF and DNA–AAFF, the fluorene ring is inserted into the helix almost perpendicular to the helix axis, whereas with DNA–AAIF, the iodine-substituted fluorene residue lies along the phosphate–sugar backbone outside the DNA helix. It is interesting from this point of view that AAF and AAFF are strong carcinogens, but AAIF apparently lacks carcinogenic activity [20].

These conformational changes cause a marked distortion of the double-stranded DNA helix at sites of AAF modification. The best evidence for localized regions of denaturation comes from the studies on the susceptibility of AAF-modified DNA to digestion by S_1 nuclease, a single-strand specific endonuclease from Aspergillus oryzae [21, 22]. The results indicate that modification of native DNA by covalent attachment of AAF residues leads to localized regions of denaturation, since the modified regions are excised by S_1 nuclease. The estimated number of base pairs destabilized by a single AAF modification is in the range of 5 to 50, depending on the extent of modification of the DNA, the length of the nuclease digestion period, and the NaCl concentration during the digestion [23]. Since attachment of AAF to G residues requires rotation of the base about the glycosidic bond and there is less hindrance to the rotation of bases in single-stranded than in double-stranded regions, it follows that single-stranded regions of nucleic acids are more susceptible to AAF modificaion than the double-stranded ones [11].

Since there are differences in the steric aspects connected with the modification of C-8 and N^2 positions of G, differences might also exist between the conformational distortions in the DNA helix associated with these two types of adducts. Following incubation of the modified DNA with S_1 nuclease, the undigested fraction was precipitated with cold ethanol [22]. This material and the released oligonucleotides present in the supernatant fraction were then separately hydrolyzed to nucleosides and analyzed by Sephadex LH-20 column chromatography (Fig. 3). The profile of the undigested fraction of the DNA demonstrates a decrease in the ratio of the C-8 to N^2 guanine AAF adducts. In addition, the C-8, but not the N^2, adduct was detected in the fraction released by nuclease S_1. Thus, the enzyme recognized the N-(deoxyguanosin-8-yl)-AAF but not the 3-(deoxyguanosin-N^2-yl)-AAF modified regions as single-stranded regions on AAF-modified DNA.

It appears, therefore, that in contrast to the C-8 adduct, substitution of AAF on the N^2 position of guanine does not produce a major change in conformation of the DNA helix. Although the precise conformation of the helix at the latter sites has not been determined, model-building studies indicate that the N^2 position, in contrast to the C-8 position of guanine, is readily susceptible to chemical modification, and the fluorene residue could simply occupy the minor

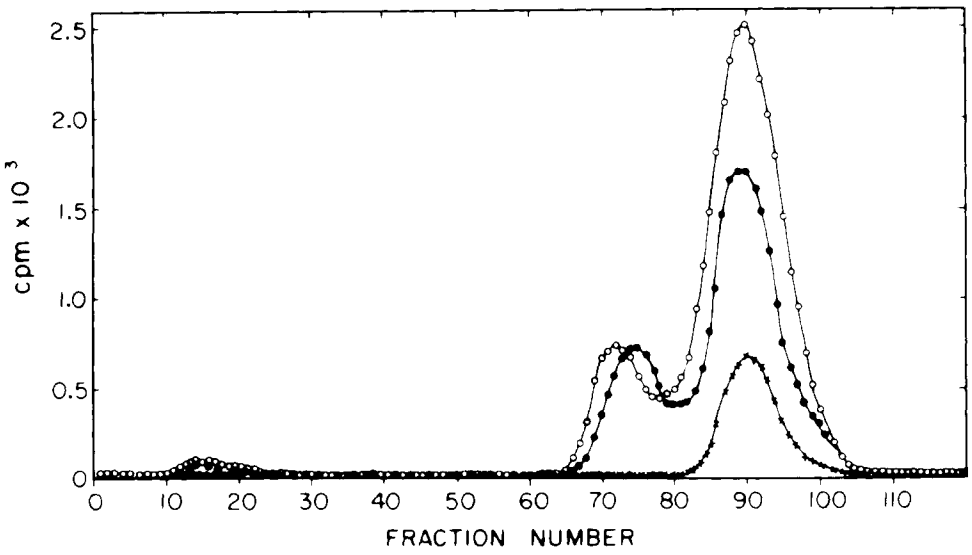

Fig. 3. Sephadex LH-20 column chromatography of nucleoside hydrolysates of the total, the Neurospora crassa nuclease-digested fraction, and the N crassa nuclease-resistant fraction of [^{14}C]AAF-modified DNA. The [^{14}C]AAF-modified DNA was completely digested to nucleosides after heat denaturation. N crassa nuclease digestion was performed in 10 mM Tris-HCl (pH 7.9), 100 mM NaCl, and 1 mM $MgCl_2$ for 3 hr at 37°C. The resistant fraction was precipitated with 2.5 vol of ethanol, collected by centrifugation, and hydrolyzed to nucleosides. (○), total [^{14}C]AAF-modified DNA; (●), N crassa nuclease-resistant fraction; (X), N crassa nuclease-digested fraction. From H. Yamasaki et al, Cancer Res 37:3756, 1977, with permission.

groove of the DNA helix. Thus, the base displacement model may apply only to the C-8 and not to the N^2-G adduct of AAF.

CONFORMATION OF AF MODIFIED DNA

After in vivo administration of N-OH-AAF, a major fraction of the DNA-bound carcinogen is N-(deoxyguanosin-8-yl)-2-aminofluorene. Because of the probable importance of this adduct in carcinogenesis, several groups have begun to investigate the conformation of AF-modified DNA.

Kriek and Spelt [23] have used nuclease S_1 digestion of DNA modified with either AF or AAF to study differences in conformation of these modified polymers. The ratios V/K_m, where V is the maxium reaction rate and K_m the Michaelis constant for the modified nucleotide (Table I), indicate that the type of lesion induced by AF substitution is cleaved much more slowly than are those induced by AAF. These results suggest that the local regions of denaturation induced by AF substitution are smaller than those associated with AAF modification.

Results from our laboratory have also suggested differences in conformation between AF- and AAF-modified DNAs. We have studied the conformation of the modified dimer, dApdG, as a model compound for DNA [24]. The CD spectra (Fig. 4) show that attachment of AAF to the C-8 position of the deoxy-

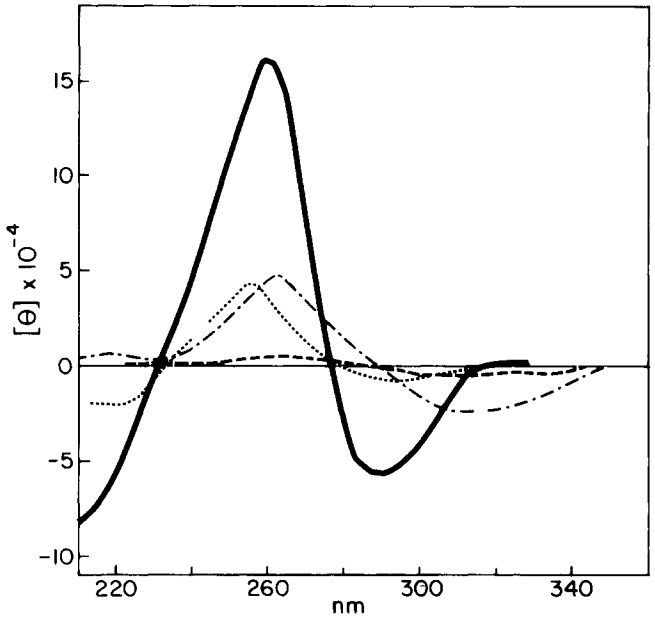

Fig. 4. CD spectra of dApdG-AAF on 0.005 M phosphate buffer (pH 7) (————) and methanol (• • • •), and of dApdG-AF in buffer (— • — • —), and methanol (— — —). From Santella et al, Carcinogenesis 1:897, 1980, with permission.

TABLE I. Rate Constants of Digestion of Carcinogen-Modified DNA by Endonuclease S_1

		Unmodified nucleotides		Modified nucleotides	
	Base subst. (%)	10^2 V/K_m (h^{-1})	K_m (mM)	10^2 V/K_m (h^{-1})	K_m (μM)
DNA-AAF	1.40	21.9	1.059	6.5	20.2
	0.78	17.7	0.665	11.8	11.2
DNA-AF	1.73	4.8	4.542	2.9	220
	0.65	4.9	2.215	2.6	284

From E. Kriek and C.E. Spelt, Cancer Lett 7:147, 1979.

guanosine residue induces large CD bands compared to the unmodified dimer. These results indicated a strong intramolecular interaction between the fluorene and adjacent base. With AF binding, the induced CD bands are only about one-third those seen in dApdG-AAF and suggests that there is some interaction between the AF residue and adjacent base, but there is less stacking than with AAF. The spectra in methanol are also shown in Figure 4. With both samples, a large decrease in [θ] value occurs indicating diminished interaction between the fluorene residue and adjacent bases. Similar results are seen with increasing temperature which like methanol is known to break up stacking interactions.

The 360 MH_z NMR spectra also indicate different interactions. Figure 5 shows the NMR spectra of the aromatic and H-1′ protons of dApdG, dApdG-

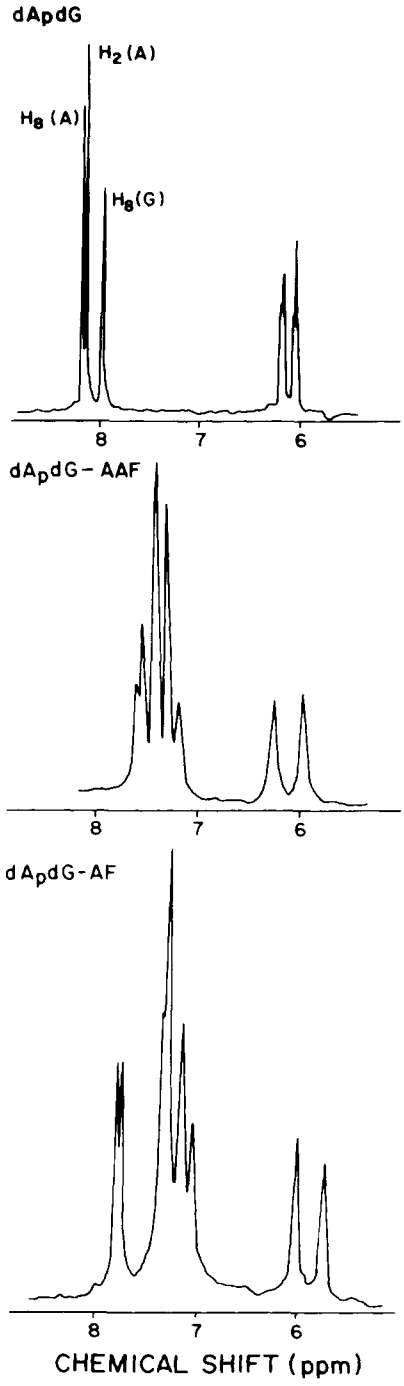

Fig. 5. Proton magnetic resonance spectra of the aromatic and H-1′ protons of dApdG, dApdG-AAF, and dApdG-AF. Chemical shifts are in parts per million downfield from the internal standard, dimethyl silapentane sulfonate. The solvent is 0.005 M deuterated phosphate buffer in D_2O at pH 7.4. From Santella et al, Carcinogenesis 1:897, 1980, with permission.

AF, and ApdG-AAF. There are upfield shifts of approximately 0.60 ppm for both the H-2 and H-8 protons of adenine with AAF binding. These shifts were attributed to a stacking interaction between the fluorene residue and adenine. In the AF-modified samples, the shift is only 0.44 ppm indicating less stacking interaction occurs than in AAF-modified samples. The temperature effects on the NMR spectra are shown in Figure 6. There is a small change in the chemical shifts of the H-8 and H-2 protons of adenine with increasing temperature for dApdG. For the AAF-modified dimer, there is a large downfield shift of these protons indicating a disruption of the stacking interaction with increasing temperature. For the AF sample, some downfield shift was seen with increasing temperature, however, at 70°C the spectra indicated decomposition of the sample.

While modification with AAF causes large distortions of the DNA as a result of rotation of the guanosine residues from the preferred *anti* to *syn* conformation, such large conformational changes may not be necessary when the bulky acetyl group is not present. This was suggested by space-filling models since in dApdG-AF rotation of the guanine from *anti* to *syn* is not required. Experimental evidence for this was obtained from NMR studies of AF- and AAF-modified guanine and GMP [25, 26]. Coupling constants for the ribose protons in the AAF-modified sample are in agreement with a model where the predominant conformation is *syn*. In contrast, similar studies for AF-modified samples suggest a predominance of the *anti* conformation. These differences in the conformation

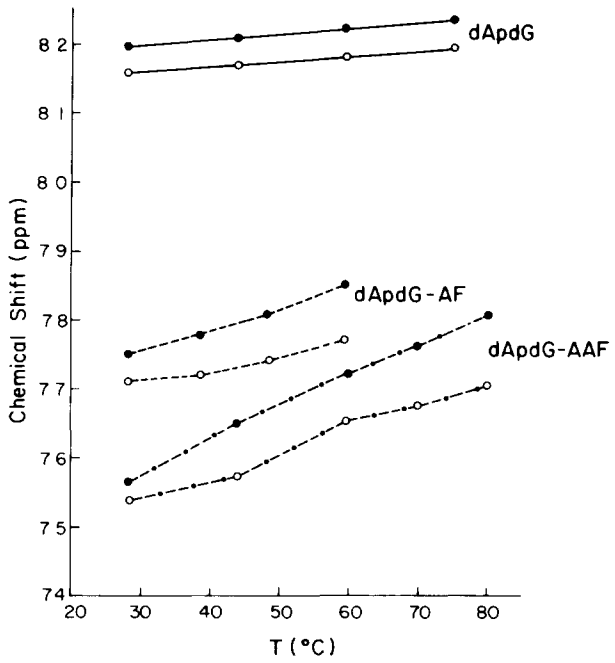

Fig. 6. Temperature dependence of the proton magnetic resonance spectra of the H-8 and H-2 protons of dApdG, dApdG-AAF, and dApdG-AF. H-8 (●), and H-2- (○) of adenine, dApdG (————), dApdG-AF (— — —), dApdG-AAF (— ● — ● —). From Santella et al, Carcinogenesis 1:897, 1980, with permission.

of monomers and dimers could be indicative of differences in conformation of these adducts in DNA, but additional studies are necessary.

Sage et al [27] have used antibodies against cytidine and adenosine to study differences in conformation between AF- and AAF-modified DNA. Denatured DNA and DNA-AAF react equally well with anticytidine antibodies in agreement with previous studies showing local regions of denaturation induced by AAF. In contrast to these results, DNA-AF reacts weakly with anticytidine antibodies indicating little denaturation of the DNA. This is in good agreement with the nuclease S_1 data discussed previously which also indicate less denaturing of the DNA with AF than with AAF modification. The results with antiadenosine antibodies are surprising, since they showed that whereas denatured DNA reacts well with the antibody, DNA-AAF and AF do not react. Previous studies [23] have shown a 5- to 50-base pair region of denaturation with AAF modification. One would, therefore, expect some reactivity with this antibody since the size of the denatured region is sufficient for reaction with the anticytidine antibodies.

Differences in reactivity of DNA-AF and AAF were also shown using antibodies to DNA-AAF or Guo-AAF [28].

CONFORMATION OF AAF-MODIFIED POLY (dG-dC)•Poly (dG-dC)

A new double-stranded conformation of deoxynucleic acids, designated Z-DNA, has been determined from crystalographic studies on the hexanucleotide $d(G-C)_3$ [29]. Unlike B-DNA, the structure has a left-handed rather than a right-handed helical sense (Fig. 7). The conformations of the guanine and cytosine deoxynucleotides differ so the helical repeat unit is made of dinucleotides rather than the mononucleotides seen in B-DNA. Figure 8 shows that the bases lie much more on the outer surface of the helix as compared to B-DNA, and that as a direct consequence of the *syn* conformation of the deoxyguanosine residues in Z-DNA the C-8 position is exposed on the outer surface of the molecule. Modification of this position may, therefore, not require such drastic distortion of the conformation as is seen in B-DNA where the C-8 position is crowded inside the helix. Additional studies have shown that the Z-DNA structure is not confined to poly(dG-dC)•poly(dG-dC), but can occur in other DNAs with alternating purine-pyrimidine sequences [30].

These studies have been related to earlier work by Pohl and Jovin [31] on the CD of poly(dG-dC)•poly(dG-dC). They showed two distinct forms in solution and a cooperative transition between them that occurs at high-salt concentrations. The CD spectrum of the high-salt form is an inversion of that of the low-salt (B-DNA) form. High ethanol concentrations gave similar results [32].

Since AAF modification of deoxyguanosine in DNA causes the rotation of the guanine from the *anti* to the *syn* conformation, a similar change in conformation could be seen in poly(dG-dC)•poly(dG-dC) with AAF modification. This might be enough to force the conformation of the entire polymer into the Z form. We, therefore, studied the CD spectra of poly(dG-dC)•poly(dG-dC) with AAF modification [33].

Figure 9 shows the CD spectra of poly(dG-dC)•poly(dG-dC) in buffer and 60% ethanol. In aqueous solution, a B-DNA spectrum is seen, whereas in ethanol the CD is inverted and characteristic of Z-DNA. Also shown is a sample modi-

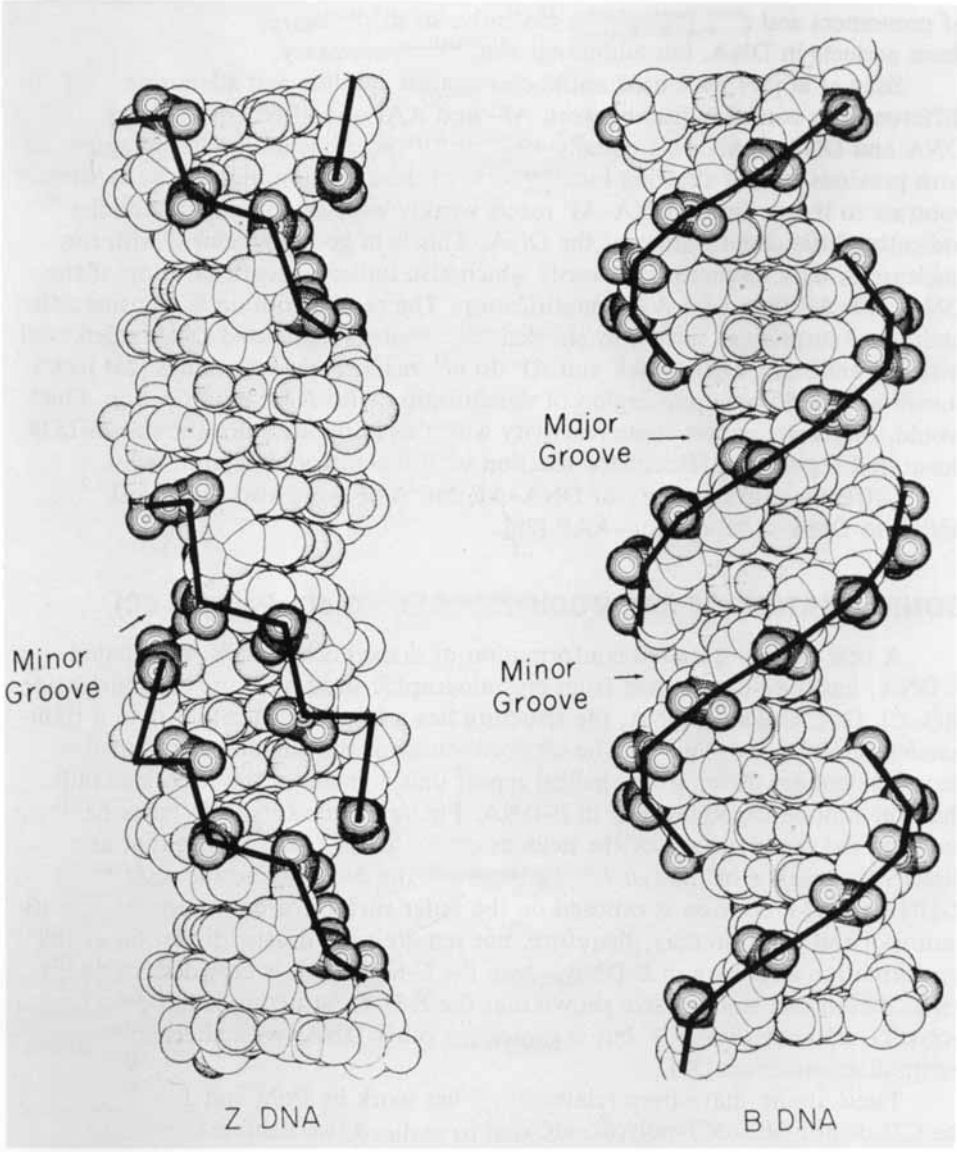

Fig. 7. Van der Waals sideviews of Z-DNA and B-DNA. The irregularity of the Z-DNA backbone is illustrated by the heavy lines which go from phospate to phosphate residues along the chain. The minor groove in Z-DNA is quite deep extending to the axis of the double helix. In contrast, B-DNA has a smooth line connecting the phosphate groups and two grooves, neither one of which extends into the helix axis of the molecule. From Wang et al, Nature (London) 282:680, 1979, with permission.

fied to the extent of 28% with AAF. With this high level of modification, the CD spectrum of poly(dG-dC)•poly(dG-dC)-AAF resembles that of Z-DNA. With lower levels of modification (3%), the sample still shows a CD characteristic of B-DNA, but is converted to that of Z-DNA at lower ethanol concentration than for unmodified poly(dG-dC)•poly(dG-dC). Sage and Leng [34] have also

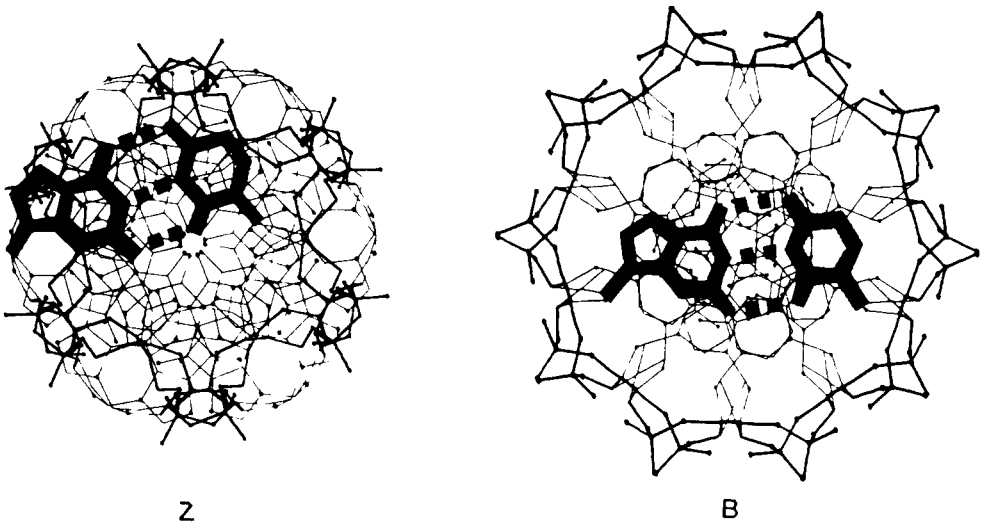

Fig. 8. End views of the regular idealized helical forms of Z- and B-DNA. Heavier lines are used for the phosphate-ribose backbone. A guanine-cytosine base pair is shown by shading. The difference in the positions of the base pairs is quite striking; they are near the center of B-DNA but at the periphery of Z-DNA. From Wang et al, Science 211:172, 1981, with permission.

shown that with low levels of AAF modification, the polymer undergoes the B to Z transition at lower ethanol concentrations. On the other hand, studies on poly(dG)•poly(dC), a homopolymer which cannot undergo the B to Z transition [31], did not show any changes in the CD spectra with high levels of AAF modification [33]. Since previous studies [35] suggested that inverted CD spectra could be a result of poly(dG-dC)•poly(dG-dC) denaturation, additional experiments were performed to exclude this possibility. Susceptibility of the modified polymers to single-strand specific nuclease S_1 and reactivity of the polymers with anticytidine antibodies were examined. The susceptibility of the polymers to nuclease S_1 digestion showed (Table II) that denatured DNA, AAF-modified DNA, AAF-modified poly(dG)•poly(dC) were susceptible to digestion with nuclease S_1 and, therefore, contain significant single-stranded regions. In contrast, poly(dG-dC)•poly(dG-dC) modified heavily with AAF was essentially resistant to nuclease S_1 digestion and must be double stranded, proving that AAF modification did not induce localized regions of denaturation in this alternating purine-pyrimidine polymer.

Similar results were obtained by using anticytidine antibodies which react only with cytidine residues in single-stranded regions of a polymer. Thus, these antibodies reacted with AAF-modified DNA [27], but not with modified poly(dG-dC)•poly(dG-dC) (unpublished data).

Taken together, the data with nuclease S_1 digestion and anticytidine antibodies seem to be in accord with the suggestion that the inverted CD spectra are indicative of a Z-DNA-type conformation. The results summarized in Table III indicate two different conformations, base displacement and Z-DNA, depending on the presence of AAF-modified deoxyguanosine residues in random or in alter-

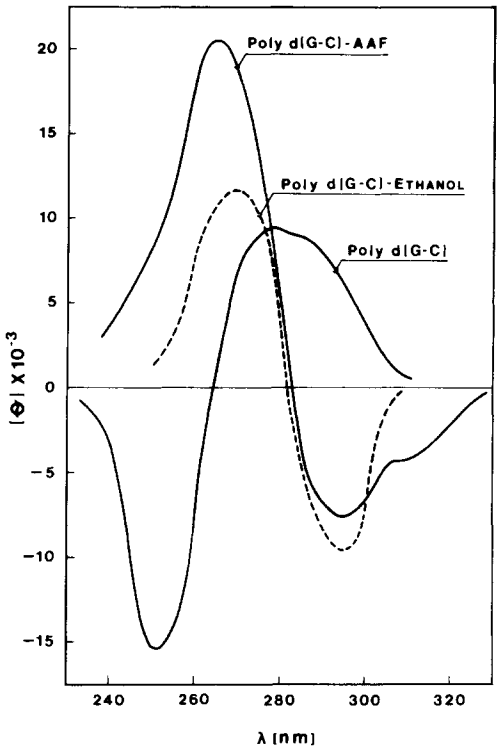

Fig. 9. CD spectra of poly(dG-dC)•poly(dG-dC). In 1 mM phosphate buffer, in 60% ethanol, and modified by AAF to an extent of 28% in 1 mM phosphate buffer. From Santella et al, PNAS 78:1451, 1981, with permission.

nating purine-pyrimidine sequences. In random sequence DNA, modified by AAF, the conformation is best represented by the base displacement model which involves the disruption of base pairing and intercalation of the AAF residue. This partial denaturation of the DNA was detected by heat denaturation [36], increased susceptibility to digestion by nuclease S_1 [21, 22], and increased reactivity with anticytidine antibodies [27]. The CD spectrum of AAF-modified DNA is essentially that of B-form DNA [36]. In contrast, alternating purine-pyrimidine sequences when modified by AAF adopt the Z conformation. Thus, modified poly(dG-dC)•poly(dG-dC) shows a CD spectrum characteristic of the Z-form DNA. Although the deoxyguanosine residues adopt the *syn* conformation as in the base displacement model, the base pairing remains intact in Z-DNA. This is indicated by the resistance of modified poly(dG-dC)•poly(dG-dC) to digestion with S_1 nuclease and the lack of reactivity with anticytidine antibodies. Although there is as yet no evidence for the existence of Z-DNA in vivo, stretches of alternating purine-pyrimidine sequences do occur in certain naturally occurring DNAs [37]. Our results suggest that if such a region were modified by AAF, this would favor its transition to the Z form. This change in conformation could induce important functional changes in that region of the genome.

TABLE II. Nuclease S_1 Digestion

	Modification (%)	Digestion (%)	
		(A_{260})	Based on (cpm)
Native DNA	0	5	
Denatured DNA	0	92	
AAF-modified DNA	20	75	83
AAF-modified poly(dG-dC)•poly-(dG-dC)	28	11	3
AAF-modified poly-(dG)•poly(dC)	19	59	54

From Santella et al, PNAS 78:1451, 1981.

TABLE III. Comparison of Properties of AAF-Modified DNA and Poly(dG-dC)•Poly(dG-dC)

	Type of AAF-modified polymers	
Properties	DNA	[Poly(dG-dC)• poly(dG-dC)]
Conformation	Base displacement	Z-DNA
CD spectra	B-DNA type	Inverted B-DNA
Base pairing	Disrupted	Intact
Nuclease S_1 susceptibility	Sensitive	Resistant
Anticytidine antibodies	Reactive	Nonreactive

CONCLUSION

Relevant to the in vivo significance of the alternative conformations of modified DNA, it is of interest that the acetylated C-8 adduct is rapidly removed from rat liver in vivo [6]. Similarly, Howard et al [38] have shown that in primary cultures of rat hepatocytes, the guanine C-8-AAF adduct is rapidly removed with a half-life of approximately 10 hr. However, the dG-N^2-AAF and dG-C-8-AF adducts remained constant for 14 hr and then were removed at a slow rate.

Taken together, these in vivo experiments and our in vitro results, suggest that the DNA excision repair enzyme system preferentially recognizes and excises lesions associated with major distortions in conformation of the DNA helix. This could account for the rapid excision of the dG-C-8-AAF adducts and for slower removal of the dG-N^2-AAF and dG-C-8-AF adducts which cause less distortion in the conformation of the DNA. In other words, carcinogen potency may depend not only on the ability of a chemical to bind to DNA and alter its template function, but also on its capacity to bind in a form that does not produce a conformational distortion that is readily recognized and excised by error-free DNA repair systems. Further studies are required to prove the validity of this assumption.

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