Z-DNA conformation of N-2-acetylaminofluorene modified poly(dG-dC).poly(dG-dC) determined by reactivity with anti cytidine antibodies and minimized potential energy calculations

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ABSTRACT

The conformation of poly(dG-dC).poly(dG-dC), poly(dG).poly(dC), and calf thymus DNA modified with N-acetoxy-N-2-acetylaminofluorene (N-acetoxy-AAF) was examined by extent of reaction with anti cytidine antibodies. In contrast to modified poly(dG).poly(dC) and DNA, modified poly(dG-dC).poly(dG-dC) failed to react with the antibodies indicating that the base pairing in this polymer is intact. This is consistent with induction of the Z-DNA conformation in AAF modified poly(dG-dC).poly(dG-dC). Using minimized potential energy calculations on the dCpdG-AAF dimer as a model for the modified polymer, it is shown that the proposed Z-DNA conformation is energetically stable. A model is proposed for an AAF modified tetramer, dGpdCpdGpdC, in which the AAF is external to the Z-DNA duplex.

INTRODUCTION

Previous studies in this and other laboratories (1,2) have elucidated aspects of the conformation of DNA modified by the carcinogen N-2-acetylaminofluorene (AAF). Binding of AAF to the C-8 position of deoxyguanosine results in a distortion of the DNA helix in which the dG residue adopts the syn conformation and the AAF residue is inserted into the helix in the position of the displaced guanine residue. This model was therefore referred to as the base displacement (3) or insertion-denaturation model (4).

With the discovery of unusual nucleic acid structures (5,6) in alternating purine-pyrimidine sequences, termed Z-DNA, in which the guanosine residues also adopted the syn conformation, we began to investigate the conformation of the synthetic copolymer poly(dG-dC).poly(dG-dC) modified with AAF. In a previous study (7), we showed that modification of poly(dG-dC).poly(dG-dC) to high levels (>20%) with AAF resulted in an inversion of the circular dichroism (CD) spectra similar to that seen in the unmodified polymer in high salt or ethanol solutions (8,9). This change in CD spectra has been attributed to a change in conformation from the B to the Z form (5,24).

Susceptibility to S_1 nuclease digestion also suggested differences in conformation between modified forms of DNA and poly(dG-dC)·poly(dG-dC). While modified DNA was sensitive to S_1 digestion, modified poly(dG-dC)·poly(dG-dC) was almost completely resistant (7,25).

Since a previous study has shown that denatured poly(dG-dC) also has an inverted spectrum (10), such changes in the CD spectra are not definitive evidence for the duplex Z form DNA. To obtain additional evidence against the possibility that our CD results are due to denaturation of poly(dG-dC)·poly(dG-dC) with AAF modification, its conformation was further investigated using anti cytidine antibodies. Since these antibodies react only with bases in single stranded regions of a polymer, they can be used to detect the extent of denaturation. For the visualization of the altered conformation of the AAF modified polymer, potential energy calculations were performed and a model is proposed.

MATERIALS & METHODS

Chemicals

Poly(dG-dC)·poly(dG-dC) and poly(dG)·poly(dC) were purchased from P-L Biochemicals, Milwaukee, WI USA; anti cytidine antibodies (11) were obtained from Dr. Bernard Erlanger, Columbia University, New York, NY, USA; and goat anti rabbit IgG was obtained from Miles Laboratories, Elkhart, IN, USA. Tritiated DNA was prepared from transformed rat epithelial W-8 cells incubated with [3 H]thymidine for 48 hours in thymidine-free media. The final specific activity was 1.9 Ci/mole and the sample was denatured by boiling, followed by rapid cooling.

Modification of Polymers With N-Acetoxy-N-2-Acetylaminofluorene

The synthetic polydeoxynucleotides and calf thymus DNA were treated with N-AcO-AAF in 1 mM phosphate buffer (pH 7.5). Nucleic acids (0.25 mg) were incubated with N-AcO-AAF (0.2 mg) for three hours at 37°C followed by extraction of the unbound carcinogen with ether. The extent of modification was determined from the ratio $A_{305}:A_{260}$ (7).

Radioimmunoassay

The radioimmunoassay was carried out at nonequilibrium condition (12). Anti C antibodies diluted 1:25 (0.1 ml) were incubated with various concentrations of the inhibitor, unlabeled dDNA or modified nucleic acid (0.1 ml), for two hours at 37°C in 0.01 M TRIS (pH 7.8). After cooling to 4°C for 10 minutes, the tracer of [3 H]dDNA (0.1 ml $\approx$ 25,000 cpm) was added, followed five minutes later by goat anti rabbit IgG (0.1 ml of

a 1:10 dilution). The samples were kept at 4°C for 45 minutes, centrifuged, and the precipitates dissolved in 0.1 M NaOH for counting. The concentration of diluted antibodies was chosen such that 12,000 cpm were bound in the absence of an inhibitor and this value was taken as 100%. Blank samples of about 1,000 cpm obtained in the absence of anti C antibodies were subtracted from each assay.

Potential Energy Calculations

Calculations were made for the AAF adduct with dCpdG (Fig. 1) considering contributions to the energy from Van der Waals, electrostatic, torsional, and deoxyribose strain terms as in earlier work (13). In addition, the potentials for the DNA employed previously were improved according to the method of Srinivasan and Olson (14), with anomeric potentials for phosphate and deoxyribose, and phosphate charge counterion and screening terms. The twelve torsion angles designated in Figure 1 and the pucker of both deoxyriboses were flexible parameters, and the energy was minimized as a function of these fourteen variables. Starting conformations were all the ω', ω, ψ combinations of dCpdG (15) and other deoxydinucleoside monophosphates (13) below 5 kcal/mole, together with the four C(3')-endo and C(2')-endo sugar pucker combinations. Plausible starting values of $\pm 45^\circ$ for α and β , 0° and 180° for γ , 0° for δ , and glycosidic torsions anti for cytidine and syn for guanine were used. Details of the calculations are to be published elsewhere (15). Some preliminary results have been presented (16).

RESULTS & DISCUSSION

In our previous study (7), we have shown that S_1 nuclease cannot detect any significant single stranded regions in AAF modified poly(dG-dC)·poly(dG-dC). Another very sensitive method for the detection of denatured sites in a double stranded polymer is radioimmunoassay using anti cytidine antibodies which react only with cytidine residues in single stranded regions.

In the absence of an inhibitor, these antibodies are able to precipitate a tracer of [^3H] denatured (dDNA), but not native DNA (11). Addition of unlabeled dDNA inhibits the precipitation of the radioactive tracer. This is shown in Figure 2 in which the inhibition of precipitation of [^3H]dDNA by anti C antibodies is shown as a function of the concentration of the various inhibitors. As shown previously (17), the inhibition of precipitation of [^3H]dDNA by dDNA or DNA-AAF

Figure 1. Structure, numbering scheme, and variable conformational angle designation for dCpdG-AAF. The dihedral angles A-B-C-D are defined as follows:

χ : 01'-C1'-N1-C6	ω : C5'-O5'-P-03'
χ' : 01'-C1'-N9-C8	α : N9-C8-N-C2
ψ, ψ_1 : C3'-C4'-C5'-O5'	β : C8-N-C2-C1
ϕ' : P-03'-C3'-C4'	γ : C8-N-C-Cm
ϕ : C4'-C5'-O5'-P	δ : N-C-Cm-H
ω' : O5'-P-03'-P	

The angle A-B-C-D is measured by a clockwise rotation of D with respect to A, looking down the B-C bond. A eclipsing D is 0°. Sugar pucker in the calculations is defined by the pseudorotation parameter (23). ϵ_1 is 5' linked, ϵ_2 is 3' linked.

modified to an extent of 11%, are similar. This is in agreement with the base displacement model where the G-C base pairing is disrupted, resulting in the cytidine residues becoming accessible to the antibodies. Modified poly(dG-dC)·poly(dG-dC), even with 21% modification, does not inhibit the binding of the antibody to the tracer indicating that this polymer cannot itself react with the antibody and therefore must not

Figure 2. Radioimmunoassay at nonequilibrium conditions in which the binding of purified anti C antibodies to [3 H]dDNA was measured in the presence of various concentrations of dDNA (●), DNA-AAF 11% modified (○), [poly(dG-dC).poly(dG-dC)]-AAF 21% modified (□), and [poly(dG).poly(dC)]-AAF 5% modified (Δ).

contain any significant single stranded regions. In contrast, poly(dG).poly(dC) which from CD studies is unable to adopt the Z-conformation (9) shows different results than the alternating copolymer. The sample with 5% AAF modification does react with the antibodies and therefore inhibits their binding to the radioactive tracer. The level of inhibition is not as great as with the DNA-AAF with 11% modification, but this may simply indicate a greater amount of denatured regions in the more highly modified sample.

The results presented here are consistent with our previous findings that AAF modified poly(dG-dC).poly(dG-dC) adopts a conformation whose properties are similar to that of Z-DNA (7). In order to propose a possible conformation of the modified polymer, potential energy calculations were performed on the dCpdG model system.

Figure 3. Minimum energy conformation of dCpdG-AAF with DNA backbone torsion angles similar to dCpdG segment of Z-DNA (18). Values of the torsion angles in a) are: $\chi'=40^\circ$, $\phi'=28^\circ$, $\omega'=90^\circ$, $\omega=53^\circ$, $\phi=195^\circ$, $\psi=173^\circ$, $\chi=252^\circ$, $P_1=48^\circ$, $P_2=180^\circ$, $\alpha=96^\circ$, $\beta=-67^\circ$, $\gamma=-1^\circ$, $\delta=63^\circ$; b) values of torsion angles are almost identical but α and β which characterize the orientation of the fluorene are 44° and 48° , respectively.

Of special interest to the present study are a representative pair of low energy local minimum energy conformations whose DNA backbones are alike and very similar to that of the dCpdG segment of Z_I-DNA (18). Figure 3a and b show ORTEP (19) drawings of these computed dCpdG-AAF adduct conformers. The guanine is syn and approximately coplanar with cytidine, while the AAF residue is twisted nearly perpendicu-

lar to the G, the deoxyriboses are alternately C(3')-endo (G) and C(2')-endo (C) domains, the O3'-P and O5'-P rotations are both gauche⁺, and the in chain C4' -C5' torsion is trans in both these forms. The two conformers differ in the orientation of the fluorene, characterized by torsion angles α and β which are flexible because of its unimpeded location at the helix exterior. In Figure 3a the α and β are 96° and -67°, respectively, while in Figure 3b they are 44° and 48°. A number of conformers with a similar backbone but differing in α and β have been computed. Their energies are in the range of 3-5 kcal/mol. The energy minimized structures shown in Figure 3a and b readily fit into the Z_I-DNA double helix. Figure 4 shows the computed adduct of Figure 3b inserted into a model of the Z_I-DNA tetrameric duplex, dGpdCpdGpdC, in the place of a dCpdG segment. The AAF is located on the outside of the double helix and induces little strain or distortion. Judging from the model, the long axis

Figure 4. Minimum energy conformation of dCpdG-AAF shown in Figure 3b inserted into a Z_I-DNA tetrameric duplex of dGpdCpdGpdC. The AAF is linked externally to the Z-DNA helix and is mobile, with rotational flexibility about its long axis.

of the fluorene makes an angle of approximately 35° with the helix axis.

Based on these and on our previous results (3,7), two different conformations are suggested for modified polymers, base displacement, and Z-DNA. 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 (4), increased susceptibility to digestion by S_1 nuclease (20,21) and increased reactivity with anti C antibodies (17). The CD spectrum of AAF modified DNA is essentially that of B form DNA (4). 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 anti C antibodies.

The biological implications of the Z-DNA conformation are not yet clear. However, since in certain naturally occurring DNAs, stretches of alternating purine-pyrimidine sequences are present (22), we can assume that Z-DNA may also exist in vivo. Because in Z-DNA the base pairs are located at the periphery of the molecule (18), the C-8 position of G residues in the syn conformation may be more exposed to attack by AAF. In such a case, these regions might be more susceptible to modification by carcinogens. Further studies are required to confirm these suggestions.

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REFERENCES

1. Grunberger, D., and Weinstein, I.B. (1979), in Chemical Carcinogens and DNA, Grover, P.L. (ed.), CRC Press, Boca Raton, FL, pp. 59-94.
2. Fuchs, R., and Daune, M. (1974), Biochemistry 13, 4435-4440.
3. Grunberger, D., and Weinstein, I.B. (1976), in Biology of Radiation Carcinogenesis, Yuhas, J.M., Tennant, R.W., & Regan, J.D. (eds.), Raven Press, New York, pp. 175-187.
4. Fuchs, R.P.P., and Daune, M. (1972), Biochemistry 11, 2659-2666.
5. Wang, A.H., Quigley, G.J., Kolpak, F.J., Cranford, J.L., Van Boom, J.A., Van der Macel, G., and Rich, A. (1979), Nature 282, 680-686.
6. Arnott, S., Chandrasekaran, R., Birdsall, D.L., Leslie, A.G.W., and Ratliff, R.L. (1980), Nature 283, 743-745.
7. Santella, R.M., Grunberger, D., Weinstein, I.B., and Rich, A. (1981), Proc. Natl. Acad. Sci. USA 78, 1451-1455.
8. Pohl, F.M. (1976), Nature 260, 365-366.
9. Pohl, F.M., and Jovin, T.M. (1972), J. Molec. Biol. 67, 375-396.
10. Narasimhan, V., and Bryan, A.M. (1976) Biochem. Biophys. Acta 435, 433-437.
11. Erlanger, B.F., and Beiser, S.M. (1964), Proc. Natl. Acad. Sci. USA 52, 433-437.
12. Poirier, M.C., Yuspa, S.H., Weinstein, I.B., and Blobstein, S. (1977), Nature 270, 186-188.
13. Broyde, S., Wartell, R., Stellman, S., and Hingerty, B. (1978), Biopolymers 17, 1485-1506.
14. Srinivasan, A., and Olson, W. (1980), Fed. Proc. 39, 2199.
15. Hingerty, G., and Broyde, S., to be published.
16. Broyde, S., Stellman, S., and Hingerty, B. (1981), in Proc. 2nd SUNYA Conversation in the Discipline Biomolecular Stereodynamics, I, R.H. Sarma (ed.), Adenine Press, New York.
17. Sage, E., Spodheim-Maurizot, M., Leng, M., Pascale, R.I.O., and Fuchs, R.P.P. (1979), FEBS Lett. 108, 66-68.
18. Wang, A.H.J., Quigley, G.J., Kolpak, F.J., Van der Marel, G., Van Boom, J.H., and Rich, A. (1980), Science 211, 171-176.
19. ORTEP: A Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations, Carroll K. Johnson, Oak Ridge National Laboratory, Oak Ridge, TN, U.S.A.
20. Fuchs, R.P.P. (1975), Nature 257, 151-152.
21. Yamasaki, H., Pulkrabek, P., Grunberger, D., and Weinstein, I.B. (1977), Cancer Res. 37, 3756-3760.
22. Astell, C.R., Smith, M., Chow, M.B., and Ward, D.C. (1979), Cell 17, 691-703.
23. Altona, C., and Sundaralingam, M. (1972), J. Amer. Chem. Soc. 94, 8205-8212.
24. Sage, E., and Leng, M. (1980), Proc. Natl. Acad. Sci. U.S.A. 77, 4597-4601.
25. Sage, E., and Leng, M. (1981), Nucleic Acid Res. 9, 1241-1250.