AAF linked to the guanine amino group: a B-Z junction

B.Hingerty

Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, and

S.Broyde

Biology Department, New York University, New York, NY 10003, USA

Received 17 January 1983; Revised and Accepted 18 April 1983

ABSTRACT

Minimized conformational potential energy calculations have been performed for AAF linked to dCpdG at the guanine amino group. This is a model for the minor AAF adduct observed in DNA, whose conformational influence has been difficult to ascertain. A global minimum energy conformation was computed with torsion angles like those of the dCpdG residue of Z-DNA. This conformation was incorporated into a larger polymer model at a B-Z junction, with the carcinogen residing in the groove in the Z direction. Local minimum energy conformations of the B type were also computed. In addition, two minima were found with fluorene-cytidine stacking. These results suggest that existing B-Z junctions may be vulnerable to modification by AAF at the guanine amino group, or that such junctions may be induced by the carcinogen if the base sequence is appropriate. Otherwise the carcinogen can be located in the minor groove of the B helix (5,10,11) or covalently intercalated (13-15).

INTRODUCTION

In an effort to understand the conformational influence of the model polycyclic aromatic carcinogen N-2-acetylaminofluorene (AAF) on DNA conformation, we have performed minimized conformational potential energy calculations for the deoxydinucleoside monophosphate dCpdG, modified by AAF and derivatives. Dinucleoside monophosphates are the smallest subunits of DNA which contain all the torsional variables of the larger polymer; hence, they are the DNA conformational building blocks and are good models to investigate. At the same time, when modified with AAF, as many as 15 simultaneously flexible rotations must be considered, making dinucleoside monophosphates the largest subunits that can practically be investigated by a global conformation

search employing minimized potential energy calculations. Since no crystal structures or detailed NMR analyses are available for such carcinogen-DNA adducts, the theoretical delineation of conformation is of particular importance. In previous work we have computed the conformations of the AAF adduct to DNA (1,2) which links to C-8 of guanine via the amino group of AAF, using dCpdG as a model. We have also studied the same adduct in its deacetylated (AF) form (3,4).

Because the C-8 adducts represent the overwhelming majority of DNA adducts in vivo and in vitro, it has been possible to study them extensively. The guanine C-8 AAF adduct causes extensive damage; it is also largely repaired in vivo (5). The C-8 linked deacetylated AF adduct appears to be partially repaired in vivo (6,7).

A second adduct site, between guanine N² and C-3 of AAF has also been observed in vitro and in vivo (8). While this minor adduct represents only a small fraction of the lesions, it may be important to carcinogenesis since it persists and is not repaired (8,9). Furthermore, very little is known about its conformational influence on DNA, precisely because it is a minor constituent, and because of difficulty associated with its synthesis in DNA subunits. It has been shown that this adduct causes little DNA denaturation (10). Model building has suggested a number of possible orientations for the carcinogen, although conformational details are lacking. A model with the carcinogen in the minor groove of B-DNA has been proposed (5,10,11). A B form model, but with syn guanine at the modification site, which places the carcinogen in the major groove, has also been presented (12). In addition, covalent intercalation models have been offered for guanine N² linked polycyclic aromatic carcinogens, which feature carcinogen-base stacking (13-15).

In order to elucidate the conformational details associated with N² guanine modification of DNA by AAF, we have performed minimized conformational potential energy calculations for this adduct with dCpdG (Figure 1), dCpdG-

$N^2(G)-C-3(AAF)$. To our surprise, we found that the only local minimum energy conformations computed to 4.4 kcal./mole had DNA torsion angles like those of the dCpdG segment of Z-DNA (ω' , ω , ψ = g^+ , g^+ , t , syn guanine deoxy-

Figure 1: Structure, numbering scheme, and variable conformational angle designations for dCpdG- $N^2(G)-C-3(AAF)$. The dihedral angles A-B-C-D are defined as follows:

X' : O1'-C1'-N1-C6 (O1'-C1 -N1-C2)	α' : <u>N1-C2-N2-C3</u>
X : O1'-C1'-N9-C8 (O1'-C1 -N9-C4)	β' : <u>C2-N2-C3-C2</u>
$\psi_1, \psi(\gamma)$: C3'-C4'-C5'-O5'	γ' : <u>C3-C2-N-C</u>
$\phi'(\epsilon)$: P-O3'-C3'-C4'	δ' : <u>C2-N-C-Cm</u>
$\phi(\beta)$: C4'-C5'-O5'-P	ϵ' : <u>N-C-Cm-H</u>
$\omega'(\zeta)$: O5'-P-O3'-C3'	
$\omega(\alpha)$: C5'-O5'-P-O3'	

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 P (29). P_1 is 5' linked, P_2 is 3' linked. AAF atoms are underscored. IUPAC recommended nomenclature is given in parentheses. The IUPAC convention for χ' and χ requires 180° be added to the values reported here.

ribose pucker C-3'-endo, anti cytidine deoxyribose pucker C-2'-endo), although no starting conformations from this domain were employed (see Methods). (The designations g^+ , t and g^- represent torsion angles in the gauche⁺ (60°), trans (180°) and gauche⁻ (300°) domains, $\pm 30^\circ$.) It was not possible, for steric reasons to incorporate this conformation in a Z helix. However, the conformer fitted very nicely into a B-Z junction, placing the carcinogen in the groove in the Z direction.

METHODS

The potentials employed were identical to those described for the C-8 adduct (1). Bond lengths and angles, torsion barriers, partial charges, Lennard Jones potential parameters, dielectric constant and minimization method were also the same as for the C-8 adduct (1), except for the following assignments: (1) Partial charges for AAF and linkage site, computed as described previously by the INDO method, with a program kindly supplied by Prof. G. Underwood, are shown in Figure 2. Partial charges for the pendant 3' and 5'-OH groups now match their in-chain values at the oxygen, with the hydrogens assigned charges of 0.131 and 0.186 respectively. (2) Bond lengths chosen for the linkage site are standard values (30): C-2 - N-2 = 1.34 Å, N-2 - C-3 = 1.48 Å; N-2 - H = 1.07 Å, with trigonal nitrogen bond angles. (3) Torsion barriers for α' and β' , computed for the guanine amino adduct of AAF with deoxyguanosine by Lipkowitz (16), using the CNDO/2 method, are 8.61 and 2.68 kcal./mole respectively. Torsion barriers for γ' , δ' and ϵ' were 3.54, 20 and -1.34 kcal./mole. Starting conformations in degrees were the following combinations (see Figure 1 for torsion angle definitions) χ' , χ : anti 15 (C3'-endo), 55 (C2'-endo); χ : syn 240; ρ_1 , ρ_2 : 18 (C3'-endo), 162 (C2'-endo). For the ω' , ω , ψ combination all the minimum energy regions of dCpdG (Hingerty and Broyde, unpublished) and other deoxydinucleoside monophosphates below 5 kcal./mole (17) were investigated. These are $\psi = 60$, ω' , $\omega = 60$, 60; 60, 180; 60, 290; 180, 290; 290, 180; 290, 290; $\psi = 180$,

Figure 2:
Partial charges
computed for AAF
and guanine amino
group linkage
vicinity.

ω' , $\omega = 60, 60; 180, 60; 290, 60; 290, 180; \psi = 300, \omega', \omega = 60, 290; 290, 60; 290; 290; \phi' = 200; \phi = 180; \alpha', \beta' = \pm 90; \gamma' = 180; \delta' = 180; \epsilon' = 0.$ ψ_1 was flexible but equal to ψ and was subsequently optimized for conformers below 15 kcal./mole. All 15 torsions, including both deoxyribose puckers were flexible.

Molecular models were constructed from MDI/Academic Press components. Space filling components were employed wherever possible close contacts existed.

RESULTS AND DISCUSSION

The only minimum energy conformation computed below 1.2 kcal./mole is illustrated in Figure 3. At 1.2 kcal./mole there is a similar conformer with the acetyl reversed: $\gamma' = 3^\circ$ and $\delta' = 34^\circ$. These conformers are like the dCpdG segment of Z DNA with $\omega', \omega, \psi = g^+, g^+, t$, $\phi' = g^-$, syn guanine with C-3'-endo sugar pucker and anti cytidine with C-2'-endo sugar pucker (18,19). Only variants of this

Figure 3: Z type global minimum energy conformation computed for dCpdG-N²(G)-C-3(AAF). The torsion angles in degrees are: $\chi' = 37$; $\psi_1 = 177$; $\phi' = 282$; $\omega' = 87$; $\omega = 57$; $\phi = 196$; $\psi = 177$; $\chi = 242$; $P_1 = 43$; $P_2 = 180$; $\alpha' = 188$; $\beta' = 83$; $\gamma' = 240$; $\delta' = 178$; $\epsilon'_1 = 0$.

ω' , ω , ψ combination are found to 4.4 kcal./mole. The Z like conformation was not found as the global minimum for either the C-8 adduct with AAF (1), or AF (4) or for dCpdG itself (Broyde and Hingerty, unpublished). Stabilizing interactions between the guanine amino linked AAF and the DNA backbone are responsible for the preference, as may be seen in Figure 3. A molecular model (see below) and an evaluation of the energies, revealed many small favorable Van der Waals interactions between the carcinogen and the backbone. Other syn guanine conformations to 10 kcal./mole have ϕ' , ω' , ω , ϕ , $\psi = t, g^-, g^+, t, t$; t, t, g^+, s^+, t ; t, g^-, g^-, t, g^+ ; g^-, g^-, t, s^+, g^+ ; t, s, t, s^-, g^+ ; t, g^+, t, g^+, t . For ϕ' s^+ represents skew near 240° and s^- is near 140° . For ω' , s is near 20° . The guanine deoxyribose, P_1 , prefers C-3'-endo type pucker (8 of 10 conformers), while P_2 , the cytidine deoxyribose may slightly prefer C-2'-endo (6 of 10 conformers). These conformers all retain guanine-cytidine stacking.

The global minimum suggested building a Z-DNA helix above and below the energy refined dinucleoside monophosphate adduct. However, this proved impossible because of collisions between the carcinogen and the Z DNA

Figure 4: Molecular model incorporating Z type global minimum energy conformation for dCpdG-N²(G)-C-3(AAF) into a B-Z junction. See text.

backbone in the 3' linked direction (to the deoxyribose of the modified guanine), which could not be alleviated by small torsion angle adjustments. However, there was no difficulty in adding the Z form to the C4'-C5' free end on

the deoxyribose of cytidine (see Figure 4). Z_{II} DNA, which is considered more likely than the Z_I form at a B-Z junction (18), was employed. In the 3' linked direction, a junction to the average B form in the dodecamer crystal (20) was easily constructed by changing the C4'-C5' rotation ψ from g^+ (54°) to trans (145°), and the O3'-P rotation ω' from 252° to 320° within its preferred domain of g^- . This junction was specifically designed to accommodate the bulky acetyl group of AAF. The next residue was then constructed with the average B form conformation. The opposite strand was constructed with the Z_{II} DNA conformation, a junction with C4'-C5' = g^- (290°), O3'-P = g^- (340°), and the other torsions in the average B conformation, followed by the B form. The carcinogen resides in the Z helix groove. The bulky acetyl group of AAF causes a slight bend in the helix axis. All Watson-Crick base pairs could be formed, with minor strain consistent with torsion angle adjustments in the 5 - 10 degree range. A single stranded region, indicated for a B-Z junction from S_1 nuclease digestion studies of supercoiled DNA (21), is not required in the present model, or in that of Arnott and Chandrasekaran (22) or of Sasisekharan, et al. (23). The detailed conformational features of B-Z junctions in supercoiled DNA may be different from the linear form, or the nuclease may be recognizing conformational alterations other than base pair rupturing. The Z type conformation stabilized by the carcinogen need not exist over a large number of base pairs, but can merge back into the preferred B form after the dinucleotide building block (24).

The present results are of particular interest in view of the findings that G-C and C-G sequences are mutational hotspots for AAF (the N-acetyoxy derivative of AAF) in the pBR322 plasmid (25,26). A mutation in the oncogene of human bladder carcinoma has also recently been found at a G-C-G sequence (Tabin, et al., 1982), suggesting that such sequences may be generally vulnerable to bladder carcinogens like AAF in the oncogene. The potential for even such a short alternating G-C sequence to adopt a Z like form (24),

Figure 5: B type local minimum energy conformation computed for dCpdG-N²(G)-C-3(AAF). The torsion angles in degrees are: $\chi' = 54$; $\psi_1 = 56$; $\phi' = 192$; $\omega' = 271$; $\omega = 292$; $\phi = 169$; $\psi = 57$; $\chi = 78$; $p_1 = 146$; $p_2 = 158$; $\alpha' = 173$; $\beta' = 114$; $\gamma' = 244$; $\delta' = 179$; $\epsilon' = -2$.

together with the evidence that the sequences are mutational hotspots, ties in with our calculated results and model which indicate a Z conformation at the modification site. The syn guanine in this conformation can be misread so that a mutation can ensue (12). It is possible that repair enzymes would fail to recognize this more subtle structural effect of N² linked AAF, compared to the gross distortion involving kinking and denaturation induced by C-8 linked AAF, which is repaired.

In the vicinity of 10 kcal./mole are found two additional types of interesting conformers. A B form conformation with guanine anti and stacked with cytidine is illustrated in Figure 5. Many variants of this conformer, differing in deoxyribose pucker and orientation of the carcinogen, are found between 10 and 13 kcal./mole. When incorporated in a larger B type polymer this conformation places the carcinogen in the minor groove as proposed by

previous models (5,10,11). This conformation may be important for base sequences that cannot adopt the Z form. The energy difference between the B type conformer and the Z like global minimum may be considerably less than 10 kcal./mole in larger polymers, since the carcinogen is stabilized by interactions with DNA atoms in the minor groove of the B form, at the adjacent residue which is not included in the calculation. In addition, the stabilization of the Z form global minimum by Van der Waals interactions between the carcinogen and the backbone may be diminished in real solutions where water and counterions compete. While coulombic screening and counterion condensation terms are included in the calculations (1), it is currently not possible to accurately evaluate the influence of water and salts by theoretical means. Furthermore, the B-Z junction energy, recently estimated at 7.7 kcal. per junction (31) must be taken into account. Nonetheless, the Z type conformer is an important one; it will be interesting to determine if it represents a general conformational influence of polycyclic aromatic carcinogens that link to the guanine amino group.

Two conformations with fluorene-cytidine stacking, with energies near 10 kcal./mole are shown in Figure 6. These are consistent with covalent intercalation models for N² linked carcinogens (13-15). Such fluorene-cytidine stacked forms are likely to be less favored in larger polymers than the B type conformer since the latter does not distort the normal B helix, while the carcinogen base stacked forms have altered conformations. The conformer shown in Figure 6a differs from the B form most notably in that the C4'-C5' rotation ψ is g⁻ instead of g⁺ and the guanine glycosidic torsion is syn. Crowding in the 3' linked direction makes this conformer unlikely for larger polymers. The conformer of Figure 6b is Z-like in its ω' , ω' , ψ combination of g⁺, g⁺, t. However, it differs from the Z form in the guanine glycosidic rotation which is high anti instead of syn, in ϕ' which is trans rather than g⁻, and in the deoxyribose pucker which is C-3'-endo for P₁ and O-1'-endo for P₂,

Figure 6: Fluorene-cytidine stacked conformers computed for dCpdG-N²(G)-C-3(AAF). The torsion angles in degrees for 6a are: $\chi' = 38$; $\psi_1 = 55$; $\phi' = 262$; $\omega' = 302$; $\omega = 299$; $\phi = 165$; $\psi = 289$; $\chi = 243$; $p_1 = 59$; $p_2 = 162$; $\alpha' = 303$; $\beta' = 275$; $\gamma' = 238$; $\delta' = 179$; $\epsilon' = 11$. Torsion angles in degrees for 6b are: $\chi' = 39$; $\psi_1 = 179$; $\phi' = 197$; $\omega' = 61$; $\omega = 51$; $\phi = 189$; $\psi = 168$; $\chi = 103$; $p_1 = 11$; $p_2 = 73$; $\alpha' = 298$; $\beta' = 298$; $\gamma' = 265$; $\delta' = 179$; $\epsilon' = 1$.

rather than the alternating C-3'-endo- C-2'-endo combination of the Z form. It is possible that specific conditions could stabilize this sterically feasible conformer in mixed sequence DNA.

CONCLUSION

Minimized conformational potential energy calculations for the guanine N² linked adduct of AAF with dCpdG reveal a global minimum energy conformation similar to the dCpdG

segment of Z-DNA. This conformer can be incorporated into a larger polymer at a B-Z junction, placing the carcinogen in the groove in the Z direction. These results suggest that the carcinogen may induce a B-Z junction and that such junctions may be vulnerable to carcinogen modification at the guanine amino group. B type conformers, which place the carcinogen in the minor groove, were also computed as local minimum energy conformations. In addition, fluorene-cytidine stacked conformers, suggestive of covalent intercalation, were found. These fluorene-cytidine stacked forms are conformationally different from the normal B form and are therefore less likely in larger polymers. Consequently, it is more probable that the carcinogen will reside in the minor groove of the B type helix, as suggested previously (5,10,11) when a B-Z junction is not possible. However, the carcinogen-base stacked conformations are sterically feasible and could be stabilized under specific environmental conditions. Current work is in progress to study these conformers incorporated in larger duplex DNA polymers by energy minimization.

ACKNOWLEDGMENT

This work was supported jointly by American Cancer Society Institutional Grant to New York University, IN-14V (SB), NIH Grant 1R01 CA28038-02 (SB), DOE Contract #DE-AC01-81 ER60015 (SB), and by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract W-7405-eng-26 with the Union Carbide Corporation (BH). We thank Professor Robert Shapiro for many helpful discussions. We also thank Dr. S. Neidle for pointing out that a high torsion barrier for α' is indicated by the short N6-C bond length in the analogous carcinogen-nucleoside adduct N6-(12-methylbenz[a]anthracenyl-7-methyl) deoxyadenosine (28), indicating partial double bond character for amino linked adducts. Special thanks are due to Dr. K. Lipkowitz for communicating torsion barriers calculated for α' and β' prior to publication.

By acceptance of this article the publisher or recipient acknowledges the U.S. Government's right to retain a non-exclusive, royalty-free license in and to any copyright covering this article.

REFERENCES

1. Hingerty, B. and Broyde, S. (1982) *Biochemistry* 21, 3243-3252.
2. Santella, R., Grunberger, D., Broyde, S. and Hingerty, B. (1981) *Nucleic Acids Res.* 9, 5459-5467.
3. Hingerty, B. and Broyde, S. (1982) *International Journal of Quantum Chemistry: Quantum Biology Symp* 9, 125-136.
4. Broyde, S. and Hingerty, B. (1983) *Biopolymers*, In Press.
5. Kriek, E. (1974) *Biochim. et Biophys. Acta.* 355, 177-203.
6. Beland, F., Dooley, K., and Jackson, C. (1982) *Cancer Res.* 42, 1348-1354.
7. Poirier, M., True, B'Ann and Laishes, B. (1982) *Cancer Res.* 42, 1317-1321.
8. Kriek, E. (1972) *Cancer Res.* 32, 2042-2048.
9. Howard, P., Casciano, D., Beland, F. and Shaddock, J.F., Jr. (1981) *Carcinogenesis* 2, 97-102.
10. Yamasaki, H., Pulkrabek, P., Grunberger, D. and Weinstein, I.B. (1977) *Cancer Res.* 37, 3756-3760.
11. Beland, F. (1978) *Chem. Biol. Interactions* 22, 329-339.
12. Kadlubar, F. (1980) *Chem. Biol. Interactions* 31, 255-263.
13. Drinkwater, N., Miller, J., Miller, E. and Yang, N. (1978) *Cancer Res.* 38, 3247-3255.
14. Frenkel, K., Grunberger, D., Boublik, M. and Weinstein I. (1978) *Biochemistry* 17, 1278-1282.
15. Hogan, M., Dattagupta, N. and Whitlock, J. (1981) *J. Biol. Chem.* 256, 4504-4513.
16. Lipkowitz, K., Personal Communication.
17. Broyde, S., Wartell, R., Stellman, S. & Hingerty, B. (1978) *Biopolymers* 17, 1485-1506.
18. Wang, A., Quigley, G., Kolpak, F., Van der Marel, G., Van Boom, J. and Rich, A. (1981) *Science* 211, 171-176.
19. Drew, H. and Dickerson, R. (1981) *J. Mol. Biol.* 152, 723-726.
20. Drew, H., Wing, R., Takano, T., Broka, C., Tanaka, T., Itakura, K. and Dickerson, R. (1981) *Proc. Natl. Acad. Sci. USA*, 78, 2179-2183.
21. Singleton, C., Klysik, J., Stirdivant, S., and Wells, R. (1982) *Nature* 299, 312-316.
22. Arnott, S. and Chandrasekaran, R. (1981) *Biomolecular Stereodynamics, I* (Sarma, R., Ed.) pp. 99-122, Adenine Press, New York.
23. Sasisekharan, V., Bansal, M., Bramachari, S. and Gupta, G., (1981) in *Biomolecular Stereodynamics, II*, Sarma, R. Ed., Adenine Press, New York, pp. 123-149.

24. Arnott, S. (1983) Cold Spring Harbor Symposium on Quantitative Biology: Structures of DNA. Cold Spring Harbor Laboratory Publications. Volume 47, In Press.
25. Fuchs, R.P.P., Schwartz, N. and Daune, M. (1981) *Nature* 294, 657-659.
26. Yoon, K., Shelegedin, V. and Kallenbach, N. (1982) *Mutation Research* 93, 273-283.
27. Tabin, C., Bradley, S., Bargmann, C., Weinberg, R., Papageorge, A., Scolnick, E., Dhar, R. Lowy, D. and Chang, E. (1982) *Nature* 300, 143-149.
28. Carrell, H., Glusker, J., Moschel, R., Hudgins, W. and Dipple, A. (1981) *Cancer Res.* 41, 2230-2234.
29. Altona, C. & Sundaralingam, M. (1972) *J. Am. Chem. Soc.* 94, 8205-8212.
30. Pauling, L., The Nature of the Chemical Bond, Third Edition, Cornell University Press, Ithaca, New York, 1960.
31. Nordheim, A., Lafer, E., Peck, L., Wang, J., Stollar, B.D. and Rich, A. (1982) *Cell* 31, 309-318.