Visualization of an AAF induced frameshift mutation: molecular views of base displacement in B-DNA from minimized potential energy calculations

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ABSTRACT

Energy minimized structures of base displacement in an AAF modified B-DNA dodecamer are presented. A rational search strategy, beginning with a global search of the conformation space of the modified deoxydinucleoside monophosphate, together with model building by computer graphics, has been employed. A number of different minimum energy conformations have been located which reveal base displaced structures. These show fluorene interstrand stacking, fluorene inter- and intrastrand stacking, and non-stacked fluorene situated in the denatured bulge. The local helix axis is bent to various extents in the different forms, and one or two base pairs are fully denatured. One structure of especial interest offers a molecular view that suggests how AAF can induce the -2 deletion mutation observed in AAF modified *E. coli* (18).

INTRODUCTION

The base displacement (1) or the equivalent insertion denaturation model (2) describes the conformational effect on random sequence DNA of the carcinogen 2-acetylaminofluorene (AAF) linked covalently to guanine C-8 (Figure 1). This model, developed from extensive solution studies, suggests a distorted B-DNA helix in which the modified guanine is syn and displaced from its normal position within the helix while the carcinogen is inserted, the duplex is partly denatured and the helix axis is bent. The many physical studies have indicated that base displacement may, in fact, be conformationally quite complex, with different conditions leading to different degrees of denaturation and different orientation angles of the AAF (reviewed in reference 3).

In earlier work we investigated the deoxydinucleoside monophosphate dCpdG, with AAF linked to guanine C-8, by minimized semi-empirical potential energy calculations (4). This work produced atomic resolution views of the minimum energy conformations. A number of conformational possibilities emerged at that level of analysis. A group of rotamers with carcinogen-base stacking was found, together with a second group with base-base stacking and a third group with essentially perfect Z-DNA conformation (5) (the d(CpG) residue of the Z helix possesses most of the Z form conformational features), all with guanine syn. A single guanine anti conformer was also found.

Figure 1. Structure, numbering scheme and variable conformational angle designations for the N-acetylaminofluorene (AAF) adduct to guanine C-8 in d(CpG). The dihedral angles A-B-C-D are defined as follows:
 χ' , 01'-C1'-N1-C6; χ , 01'-C1'-N9-C8; ψ , C3'-C4'-C5'-O5'; ϕ' , P-03'-C3'-C4';
 ϕ , C4'-C5'-O5'-P; ω' , 05'-P-03'-C3'; ω , C5'-O5'-P-03'; α , N9-C8-N-C2;
 β , C8-N-C2-C1; γ , C8-N-C-Cm; δ , N-C-Cm-H. 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 O°. Sugar pucker in the calculations is defined by the pseudorotation parameter P (34). IUPAC torsion angle designations are given in parentheses. In the IUPAC convention 180 degrees is added to χ and χ' .

In the present work we extend these studies to the duplex dodecamer level. We employ a combination of rational search strategies and computer graphics to obtain energy minimized molecular views of base displacement in these larger double stranded structures. Since atomic resolution data is available only for the carcinogen-base adduct G-AAF (6), the structures offered here are the first such results for base displacement in the B-DNA duplex. The AAF modified Z helix is described elsewhere (7). The structures presented are representative feasible forms; because of the multiple minimum problem and the large number of conformational variables in a duplex dodecamer, it is at present impossible to locate all minimum energy conformations for a molecule of this size.

METHODS

The same potential set of Olson and Srinivasan (8), together with parameters and minimization methods described previously (4), is employed in our new program, which computes conformations to the duplex dodecamer level. In addition, for duplexes we have implemented a hydrogen bonding function similar to that described by Olson (9). In unmodified DNA duplexes this hydrogen bonding function invariably yields properly base

paired structures from minimizations of forms whose starting conformation is close to base paired and low energy. However, because of the multiple minimum problem, base paired structures may or may not be found from high energy starting conformations. We have taken advantage of this to locate important denatured forms of carcinogen modified double stranded DNA. To determine whether or not a given residue is denatured in a modified duplex, a mathematical forcing function, F , related to that of Stellman, et al. (10) is employed to seek out base paired minimum energy conformations. For a G-C pair, with ideal hydrogen bond distances of 2.81, 2.81 and 2.87 Å (11)

$$F = W ((1 + \text{CHB1})^2 + (1 + \text{CHB2})^2 + (1 + \text{CHB3})^2 + P + (d_1 - 2.81)^2 + (d_2 - 2.87)^2 + (d_3 - 2.81)^2).$$

The CHB's are cosines of the instantaneous values of the angles about the H participating in the hydrogen bond, the d 's are instantaneous values of the hydrogen bond distances, $P = \|\mathbf{c}_1 - \mathbf{c}_2\|^2$ where $\mathbf{c}_1$ is a unit vector perpendicular to the plane of one base, and $\mathbf{c}_2$ is a unit vector perpendicular to the plane of its partner. $\mathbf{c}_2$ is parallel to $\mathbf{c}_1$ when the ideal Watson-Crick pair is formed. W is an adjustable weight, currently assigned a value of 30, which locates base paired minima to the dodecamer level. This spring-like pseudo-potential function, with units of energy in kcal/mole, is non-negative and equals zero when the hydrogen bond distances are ideal, the angles about the hydrogen participating in the hydrogen bond are the ideal values of 180° , and the planes of the two Watson-Crick base paired partners are parallel. A first minimization is carried out with this function implemented at chosen residues. This will encourage selected residues to be base paired and permit the others to be denatured. The results of this first trial are then employed in a second minimization without the forcing function, to obtain proper energies and geometries. In this stage residues that were denatured may anneal and base paired forms may rupture under the influence of the force field, so that the number of ruptured base pairs in the final conformation may differ from that in the input trial. Conformers with different numbers of disrupted base pairs are energy ranked, and selection of the favored forms is made on this basis.

The search of the conformation space of the polymer is carried out in stages, as it is not possible with current computing power to directly survey the entire polymer conformation space. Modified deoxydinucleoside monophosphates, the conformational building blocks of the larger polymers, are first studied by a global search with about 4000 trials per adduct (12). Subsequently the important forms from the dimer search are incorporated into single stranded tetramers, with adjacent sites in conformationally appropriate A (13), B (14) or Z (5) DNA conformations, and the energy minimized, to assess the effect of the adjacent sites on the dimer energy rankings. Dimer energy rankings are known to be reordered when 3' and 5' phosphates are appended (15). Finally, important dimer forms from the tetramer minimizations are incorporated in one strand of the double stranded dodecamer, d(GCGCGCGCGCGC), with AAF modification at the

Table I

Duplex Dodecamer Trials	
<u>Starting Conformation</u>	<u>Residues Where Hydrogen Bond Forcing Function Is Implemented in First Stage Minimization*</u>
Figure 2(a) or 2(b) at residues 6 and 7	100000000001
	110000000001
	110000000011
	111000000011
	111000000111
	111100000111
	111100001111
	111110001111
	111110011111
	111111001111
	111111011111
Figure 4(b) at residues 6, 7 and 8**	111110001111
	111110011111
	111111001111
	111111011111

* 1 denotes implemented, 0 denotes not implemented

** other residues in B form (11)

asterisk designated G, and all other residues in the B form (11). (Tetramers are not directly incorporated into the duplex dodecamers because preliminary trials indicated that they yielded higher energy final structures than did the dimers). The forcing function is applied in a series of trials in which 1 - 10 base pairs surrounding the lesion are permitted to denature (Table 1), the energy is minimized, and each resulting conformer is energy minimized a second time without the forcing function. In addition, computer graphics is employed to search directly by eye for novel structures, especially in single stranded trimers. When such forms are found they are subject to energy minimization, followed by incorporation in the duplex dodecamer as described above for dimers.

Calculations are carried out at the DOE Cray X-MP and Cray-2 at Livermore, CA., and on the Cray X-MP at the NSF Supercomputer Center in San Diego, CA. Computer graphics resources utilized include the Evans and Sutherland Interactive Computer Graphics System with Vax 11/780 with the program Chem-X (Molecular Design, Ltd.) maintained by the New York University Academic Computing Facility.

RESULTS AND DISCUSSION

Single Stranded Structures

Forty two minimum energy dimer conformations for d(CpG)-AAF (from reference 4, 16 and unpublished minima) were investigated in the single stranded tetramer

Figure 2. Stereo views of d(CpG)-AAF. These stereo views are prepared for viewing with crossed eyes. To view with a stereo viewer left and right images should be interchanged.

(a) AAF-cytidine stacked conformer. Torsion angles $P(C)\chi', \phi', \omega', \omega, \phi, \psi, P(G)\chi, \alpha, \beta, \gamma, \delta$ are respectively in degrees 83, 58, 257, 285, 288, 144, 58, 41, 236, 94, 305, -4, 65.

(b) Guanine-cytidine stacked conformer. Torsion angles in degrees, in the same order as in 2(a) are 33, 49, 196, 294, 284, 175, 56, 44, 236, 103, 76, -3, 75.

d(GCGC)^{*}, with AAF modification at the asterisk designated G. Two equal lowest energy conformers resulted, one with AAF at the exterior of a Z-DNA type helix, and a second with carcinogen-base stacking embedded in a B form. The Z form is presented elsewhere (7). The relevant dimer minimum energy conformation with AAF-cytidine stacking is shown in Figure 2(a), and the tetramer structure is shown in Figure 3(a). In addition, a third low energy tetramer conformation (Figure 3(b), 1.6 kcal/mole above the two lowest energy states, was computed. It is derived from the dimer conformation shown in Figure 2(b) embedded in the B form, and has only base-base stacking, with the AAF blocking the Watson-Crick base pairing sites adjacent to the lesion. The conformers of Figure 3(a) and (b) are perturbed A-B type forms with syn guanines.

The carcinogen-base stacked tetramer shown in Figure 3(a) has the AAF stacked with adjacent cytidine in one direction only, with a bend in the backbone at the modified G residue. Since no low energy forms with base-AAF-base stacking were computed from these trials we decided to search for such forms directly by computer graphics model building. For this purpose the trimer sequence d(CGC) with AAF modified G was investigated. The goal was to perturb the B form as little as possible to achieve such a structure. We started by incorporating the carcinogen-base stacked dimer of Figure 2(a)

Figure 3. Stereo view of d(GCGC)^{*} with AAF modification at asterisk designated G. See caption to Figure 2.

(a) Conformer of Figure 2(a) has been incorporated into the B form (14) and the energy minimized. Torsion angles, in degrees, are in repeating order $\phi, \psi, \rho, \chi, \phi', \omega', \omega$: 184, 60, 167, 70, 186, 264, 290, 167, 58, 74, 49, 252, 280, 286, 144, 58, 37, 235, 196, 286, 294, 173, 72, 157, 55, 186. $\alpha, \beta, \gamma, \delta$ are respectively 95, 298, -2, 57 degrees.

(b) Energy minimized conformation produced by incorporation of Figure 2(b) conformer into B form (14). Torsion angles, in degrees, in same order as in 3(a) are 184, 59, 168, 66, 181, 265, 274, 182, 50, 31, 44, 207, 305, 277, 182, 57, 42, 237, 195, 285, 294, 174, 71, 157, 57, 186. $\alpha, \beta, \gamma, \delta$ are respectively 102, 93, -5, 65 degrees.

(c) Energy minimized conformation produced from trimer of Figure 4(b) to which a G residue was added to the 5' terminus in the B form (14). Torsion angles in degrees, in same order as in 3(a) are 184, 64, 179, 10, 183, 268, 297, 164, 172, 178, 57, 265, 296, 305, 138, 58, 59, 238, 185, 170, 299, 111, 62, 138, 63, 188. $\alpha, \beta, \gamma, \delta$ are respectively 92, 299, 0, 61 degrees.

Figure 4. Stereo views of d(CGC) with AAF modification at G. See caption to Figure 2.

(a) A cytidine residue in the B form (14) has been added to the 3' terminus of the conformer of Figure 2(a) and the energy minimized. Torsion angles, in degrees, are in repeating order ϕ , ψ , P , χ , ϕ' , ω' , ω : 182, 67, 114, 59, 267, 281, 287, 136, 61, 40, 236, 195, 285, 295, 172, 73, 157, 56, 186. α , β , γ , δ are respectively 94, 300, -2, 57.

(b) Trimer of Figure 4(a) altered by rotation of ω' in the added cytidine to 150 degrees and ϕ to 90 degrees from their values of 285 and 172 degrees in 4(a), followed by energy minimization. Torsion angles, in degrees, in the same order as in 4(a) are ϕ , ψ , P , χ , ϕ' , ω' , ω : 176, 59, 160, 57, 271, 288, 302, 135, 60, 57, 239, 184, 172, 297, 112, 63, 138, 63, 188. α , β , γ , δ are respectively 92, 299, -1, 62 degrees.

into the trimer, placing the added cytidine residue in the B form and minimizing the energy. The resulting trimer is shown in Figure 4(a). Inspection of the backbone torsions suggested that rotation of the O3'-P from gauche minus to trans and of the C5'-O5' torsion from trans into a near eclipsed position, in the guanine-stacked cytidine residue, would produce cytidine-AAF-cytidine stacking. The conformer generated in this way was energy minimized, and the minimized structure, shown in Figure 4(b), proved to have the predicted stacking. A guanine residue in the B form was then added to the 5' terminus, and the energy of this tetramer was again minimized, to determine its energy relative to the other tetramers. The structure shown in Figure 3(c) resulted. Its energy is 9.7 kcal./mole above the structure of Figure 3(a).

Duplexes

In the next stage duplex dodecamer trials were carried out. d(CpG)-AAF minima shown in Figures 2(a) and (b) were incorporated in the duplex dodecamer, with all other residues in the B form. A series of trials (each consisting of two minimizations, one with

Figure 5. Stereo views of base displacement in duplex d(GCGCGCGCGC)^{*} with AAF modification at asterisk designated G of one strand. See caption to Figure 2. (a) resulted from incorporation of the conformer of Figure 2(b) in the B form (11), (b) and (c) were produced from the conformer of Figure 4(b) incorporated into the B form (11), followed by energy minimization in two stages as described in the text. The hydrogen bond forcing function was applied as follows in the first stage minimization: 111110011111, 111110111111, 111110011111 for (a), (b) and (c), respectively (see Table I). Torsion angles α , β , γ , δ are respectively in degrees (a) 117, 56, 189, 27; (b) 88, 54, 7, 246; (c) 59, 50, -1, 236. Others are within observed B-DNA ranges (14) except for some perturbations in the immediate vicinity of the modification. The modified guanines are all syn. Coordinates and torsion angles are available from the authors.

and one without the forcing function) was made for each of the two forms, with 1 - 10 base pairs, at and surrounding the lesion, permitted to be denatured in the first stage. Table I summarizes all trials. The lowest energy form from this group is shown in Figure 5(a). It is 40 kcal./mole higher in energy than the fully base paired, undeformed Z helix with AAF at the helix exterior (7), in this alternating C-G sequence. This base displaced structure has two base pairs fully disrupted, a bend in the helix axis, and interstrand stacking between fluorene and base. It is derived from the conformer of Figure 2(b). Other structures computed in this series were either very similar and of similar energy, or more denatured and 10 or more kcal./mole higher in energy.

We then turned our attention to the fluorene intrastrand stacked conformer shown in Figure 4(b). To investigate it further we embedded this energy minimized trimer into the duplex dodecamer with adjacent sites in the B form, and minimized the energy of the entire structure in two stages, applying the base pair forcing function in the first stage minimization to all base pairs except at the modified G and the one or two base pairs surrounding it (see Table I). A number of interesting forms resulted from this series of trials. Figure 5(b) shows the lowest energy conformation computed for base displacement, 16 kcal/mole above the Z form. It has only one base pair ruptured, with fluorene interstrand stacking. The carcinogen is located at a severely bent site where it protrudes out into the groove. Next in energy is a conformer 31 kcal/mole above the Z form, shown in Figure 5(c), in which the AAF is not stacked with any base, but resides in the denatured bulge formed by the two ruptured base pairs. (Two such forms, close in energy were computed from the four trials in this series). Finally, a high energy conformer, 79 kcal/mole above the Z form, of unusual interest, resulted, and is shown in Figure 6(a). This form is of special importance to the effect of AAF during replication. As may be seen from the figure, the AAF is intercalated into the helix center in a virtually classical fashion, being stacked between the planes of the bases both on the same strand and on the opposite strand. The distance between the base planes opposite the lesion has been increased to accomodate the inserted AAF. Interestingly, this is achieved in part by rotation of the glycosidic torsion angle of the guanine in the intercalated C-G sequence from anti to high anti, just as in classical intercalation (17) (and the same is true for the structure shown in Figure 5(a)). Strikingly, the intercalation by AAF has created a misalignment of base pairs that is diagrammed in Figure 6(b). The modified G-6, together with its adjacent C-7 have no pairing partners, C-5 above is paired with G-7 opposite, while C-6 and G-5 opposite are unpaired. Thus, two base pairs are fully disrupted.

Relevance to Genetic Effects

Consideration of the structure of Figure 6 in terms of replication, with the modified strand as the parent and its partner as the daughter, is particularly revealing.

Figure 6. (a) Stereo view of duplex d (GCGCGCG^{*}CGCGC) with AAF modification at asterisk designated G of one strand in a base displaced conformation that produces a misalignment of base pairs in the two strands. See caption to Figure 2. The hydrogen bond forcing function was applied as follows in the first stage minimization: 111110001111. Torsion angles $\alpha, \beta, \gamma, \delta$ are in degrees 92, 291, 7, 57, respectively. Others are largely within observed B-DNA ranges (14). The modified guanine is syn. Coordinates and torsion angles are available from the authors.

(b) Interpretive diagram of the structure in (a) showing the base pairing arrangement.

We note that residues C-7 and the adjacent AAF modified G-6 are not replicated. However, there is a compensating unpaired bulge at G-5 and C-6 of the daughter strand, so that the misalignment is rectified at the 4th residue. This probably happens because the hydrogen bond potential and forcing function as currently employed encourage

location of minimum energy conformations with base pairs between properly aligned partners. However, absent such a self correction, a frameshift mutation resulting from the -2 deletion is produced. (The -2 deletion would also result if the unpaired bulge actually occurred at replication, but were subsequently cleaved out by repair enzymes.) The conformational features of the duplex with the deletion are revealed in Figure 7, which shows the parent residues 5-12 renumbered 1-8 together with daughter residues 7-12 renumbered 1-6. -2 deletions are, in fact, prominent among the mutations observed by Koffel-Schwartz and co-workers in AAF modified *E. coli* (18). Moreover, the structure of Figure 7 may offer a molecular view of -2 deletion that is applicable to other adducts of aromatic amines to guanine C-8. We have computed (unpublished) a similar structure for the deacetylated aminofluorene (AF) adduct; the AF adduct induces frameshifts (19) that are -2 deletions in *Salmonella typhimurium* TA1538 (20), and AF induced -2 deletions are also observed in *E. coli* (21,22).

Major genetic rearrangements, associated with AAF modification in Chinese Hamster Ovary cells (23), and recently observed AAF induced point mutations (24,25), might result from forms like those of Figure 5, or Z forms, in which all base pairs are aligned. Base sequence, and the particular biological system under investigation, seem to determine the type of mutation caused by AAF.

Relevance to Experimental Studies In Vitro

It is of interest to consider the computed structures in relation to the large number of classical solution studies of AAF modified random sequence DNA (see review in reference 3 and references cited therein). It is agreed that AAF induces denaturation in duplex DNA. In addition, AAF-base stacking observed in modified single stranded dimers has led to a model in which the AAF is inserted into the duplex helix. However, there is little agreement on further conformational details, other than that the guanine is syn. Experimental findings on the number of base pairs ruptured per modified site vary widely, depending on conditions, with reports ranging from 1 to 50. Electric dichroism measurements disagree on the angle between the plane of the fluorene and the helix axis, with measurements of 80° (26) and 60° (27) reported. These results are further complicated by the observation of "hinge points" in AAF modified DNA, as indicated from reduction in DNA viscosity and radius of gyration (2). Our most favored base displaced form (Figure 5(b)) has 1 base pair fully ruptured, fluorene interstrand stacking, a bend or "hinge point" in the helix axis of roughly 45° at the site of the lesion, with the plane of the fluorene close to perpendicular to the helix axis of one arm in the bent duplex. Interestingly, the AAF in this form is buried in the groove rather than inserted within a bulge.

High resolution NMR studies, perhaps coupled with restrained energy minimization

Figure 7. A molecular view of a frameshift mutation, in stereo, is shown in (a). See caption to Figure 2. Parent residues 5-12 and daughter residues 7-12 of figure 6(a) are shown. These are renumbered as 1-8 and 1-6, respectively, in (b), an interpretive diagram of the structure in (a).

calculations, will be valuable in providing experimental details for comparison with the computed structures. Some recent NMR investigations have begun to provide such data. Evans and co-workers in their proton NMR investigation of AAF modified 5'dGMP (28) have obtained details on the AAF-base orientation. Our earlier prediction (4) of a perpendicular orientation between the planes of AAF and guanine has been verified in these studies and also in the crystal structure of G-AAF (6). ^{13}C and ^{31}P NMR analyses have yielded some conformational details of base displacement in AAF modified d(pApG) (29). Very recent studies with an AAF modified duplex (30), in which all base pairs are formed and the structure is predominantly right handed, might indicate a mini Z-helix with AAF at the helix exterior embedded in a B form. Unique structures, such as this, can be envisioned for appropriate base sequences and/or conditions. Another novel structure is the sterically feasible base displaced AAF modified Z-form model (31), suggested from types of anti-Z antibodies induced by AAF modified Z-DNA (32). A base displaced Z-type structure embedded in an overall B form is yet another possible model (33).

Ordered crystals of AAF modified duplex DNA in a base displaced conformation may prove difficult to obtain because of the conformational flexibility inherent in partly denatured structures.

CONCLUSION

The base displaced structures presented here reveal some conformations accessible to AAF modified duplex B-DNA. We find structures with fluorene interstrand stacking, fluorene intra and interstrand stacking, and fluorenes that are not stacked with base. One or two base pairs are totally disrupted in these forms and the helix axis is bent to differing degrees. In all these structures the guanine is syn at the modification site, but other torsion angles are mostly in the B-DNA ranges (14). Considering that a single hydrogen bond contributes 3 - 5 kcal./mole in energy, these and other conformers may be energetically accessible, depending on base sequence and conditions of environment; a high energy minimum such as that of Figure 7 which reveals a frameshift mutation, could become the favored form during replication where large numbers of enzymes, undoubtedly employing dozens of hydrogen bonds, interact with the DNA template. The Z-helix, with all base pairs intact and external AAF, on the other hand, is the global minimum in alternating G-C sequences (7), being preferred by 16 kcal./mole over the next lowest energy form (Figure 5(b)) in the dodecamer. Consequently, base displacement or insertion denaturation is observed only in sequences where the energetic cost of achieving the Z form exceeds the energetic cost of base displacement.

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