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An Intercalated and Thermally Stable FAPY Adduct of Aflatoxin B_1 in a DNA Duplex: Structural Refinement from ^{1}H NMR †

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SUPPORTING INFORMATION

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Details of the MD refinement

Empirical base pairing distance and planarity restraints were used as follows. For G•C base pairs *r*[cytosine N4-guanosine O6] = 2.91 ± 0.05 Å, *r*[cytosine N3-guanosine N1] = 2.95 ± 0.05 Å, *r*[cytosine O2-guanosine O6] = 2.86 ± 0.05 Å, *r*[cytosine N3-guanosine N2] = 3.65 ± 0.05 Å, and *r*[cytosine O2-guanosine O6] = 5.42 ± 0.05 Å. For A•T base pairs *r*[adenosine N6-thymidine O4] = 2.95 ± 0.05 Å, *r*[adenosine N1-thymidine N3] = 2.82 ± 0.05 Å, *r*[adenosine N1-thymidine O4] =3.63 ± 0.05 Å, and *r*[adenosine N6-thymidine O2] = 5.40 ± 0.05 Å. The value of the torsion angle for Watson-Crick base pairing was: [purine C2-purine N1-pyrimidine N3-pyrimidine C2] = 0 ± 10°. Empirical base-step distances were *r*[H8-H8] = 5.00± 0.20 Å, *r*[H6-H6] = 5.00 ± 0.20 Å, and *r*[H8-H6] = 4.80 ± 0.20 Å.

The NOE distances were partitioned into five classes based on the quality of the distances generated from MARDIGRAS. Different force constants were assigned for each class (50, 40, 30, 20, and 10 kcal $mole^{-1}Å^{-2}$ for the best defined class to the worst defined class of NOE restraints, respectively). These include 388 NOE restraints in class 1, 103 restrains in class 2, 42 restraints in class 3, 34 restraints in class 4 and 15 restrains in class 5. Hydrogen bond restraints were included to ensure Watson-Crick base pairing based on the experimental observations. A force constant of 10 kcal $mole^{-1}Å^{-2}$ was assigned to these restraints. The distances were as follows for all base pairs. For the G•C base pairs r[C(N4) - G(O6)] = 2.70 ± 0.20 Å, r[C(N3) - G(N1)] = 2.91 ± 0.10 Å, r[C(O2) - G(N2)] = 3.01 ± 0.20 Å. For A•T base pairs r[A(N6) - T (O4)] = 2.80 ± 0.20 Å AND r[A(N1) - T(N3)] = 2.95 ± 0.10 Å. It was necessary to restrain backbone torsion angle to a region derived from canonical A- and B-form DNA {148,1285}. The ranges for the angles were 265-325° for α, 160-220° for β, 27-57° for γ, 80-160° for δ, 145-205° for ε, 260-320° for ζ, and 200-270° for χ. A force constant of 10 kcal $mole^{-1}Å^{-2}$ was assigned for base planarity restraints. A torsion angle restraint was added between each base pair with a range of ± 10° in order to prevent

excessive propeller twisting. These dihedral angle restraints were assigned a force constant of 10 kcal $mole^{-1}Å^{-2}$. In addition, sugar pucker restraints for B-DNA were included with a force constant of 10 kcal $mole^{-1}Å^{-2}$.

The system was coupled to a heating bath with a target temperature of 1500 K which was reached in a 3 ps interval. This temperature was maintained for 17 ps. The molecules were then cooled to 300 K in a 3 ps period and maintained at that temperature for 25 ps. Coordinate sets were archived every 0.1 ps, and 30 structures from the last 5 ps were averaged. The average MD structures were then subjected to 500 iterations of conjugate gradient energy minimization to obtain the final structures. The weights for the NOE restraints and the additional empirical restraints were modulated throughout the simulated annealing protocol by multiplying the corresponding force constants by scaling factors. Specifically, the force constants for the four classes of NOE restraints were scaled up for 3.5 ps during the heating period to 120, 100, 80, 60 and 40 kcal $mole^{-1}Å^{-2}$ in the order of best to worst defined distances. These weights were maintained during the remainder of the heating period and for the first 2 ps of the equilibrium dynamics period, and were then scaled down to 50, 40, 30, 20 and 10 kcal $mole^{-1}Å^{-2}$ in the order of best to worst defined restraints. The dihedral angle restraints and the base-pair distance restraints were also modulated throughout the MD calculation such that their force constants were scaled up to 100 kcal $mole^{-1}Å^{-2}$ during the same period as for the NOE restraints and scaled back to 10 kcal $mole^{-1}Å^{-2}$, also at the same time as the NOE restraints.

Table 1S. Assignments of non-exchangeable protons of AFB_1-FAPY modified oligonucleotide (25 °C).

Nucleotide	H5/CH_3	H6/H8	H1'	H2'	H2''	H3'	H4'	H5', H5''
C^1	5.81	7.73	5.77	2.08	2.45	4.57	4.01	3.67
T^2	1.59	7.45	5.67	2.14	2.44	4.81	4.11	3.94, 3.97
A^3		8.29	6.13	2.55	2.82	4.96	4.34	4.05, 4.10
T4	1.44	7.27	5.91	2.27	2.55	4.84	4.23	4.09, 4.11
$^{FAPY}G^5$		8.12	5.01	1.88	2.07	4.79	4.53	3.93, n.d.
A^6		8.12	6.19	2.56	2.82	4.98	4.38	3.97, 4.09
T^7	1.22	7.15	5.92	1.95	2.44	4.98	4.38	4.11, 4.18
T^8	1.52	7.28	5.98	1.94	2.33	4.78	n.d.	4.03, 4.17
C^9	5.66	7.40	5.66	1.92	2.30	4.73	4.04	3.93, 3.98
A^{10}		8.17	6.24	2.35	2.59	4.62	4.11	3.98, 4.02
T^{11}	1.64	7.24	5.69	2.06	2.21	4.50	3.92	3.55, n.d.
G^{12}		7.86	5.28	2.61	2.68	4.87	4.20	3.84, 3.92
A^{13}		8.10	5.89	2.67	2.78	4.97	4.37	3.97, 4.41
A^{14}		7.98	6.08	2.43	2.75	4.83	4.35	4.12, 4.17
T^{15}	1.10	6.92	5.75	1.88	2.39	4.68	4.17	4.04, 3.55
C^{16}	5.42	7.31	5.81	2.16	2.24	4.73	4.13	3.96, 3.98
A^{17}		8.29	6.01	2.61	2.80	4.85	4.26	3.95, 4.04
T^{18}	1.30	7.20	5.45	1.94	2.24	n.d.	4.12	3.98, 4.12
A^{19}		8.09	6.01	2.72	2.64	4.95	4.33	4.17, 4.12
G^{20}		7.61	5.90	2.16	2.33	4.55	4.17	4.04, 4.09

Table 2S. Assignments of the non-exchangeable protons of the unmodified oligonucleotide. (25 °C).

Nucleotide	H5/CH_3	H6/H8	H1'	H2'	H2''	H3'	H4'	H5', H5"
C^1	5.62	7.57	5.56	1.95	2.29	n.d.	4.39	3.82, 3.52
T^2	1.44	7.36	5.50	2.03	2.34	n.d.	4.39	3.96, 3.82
A^3		8.14	6.02	2.46	2.69	4.79	4.19	3.95, 3.89
T4	1.15	6.84	5.38	1.69	2.10	n.d.	n.d.	n.d.
G^5		7.59	5.35	2.43	2.51	4.75	4.10	n.d.
A^6		7.92	5.96	2.40	2.67	4.75	4.22	3.89, 3.85
T^7	1.03	6.94	5.69	1.74	2.29	n.d.	4.57	3.96, 3.92
T^8	1.33	7.13	5.82	1.79	2.19	n.d.	n.d.	n.d.
C^9	5.46	7.24	5.45	1.76	2.04	4.57	n.d.	n.d.
A^{10}		8.02	6.07	2.20	2.46	n.d.	4.46	3.95, 3.83
T^{11}	1.35	7.09	5.49	1.19	1.93	n.d.	n.d.	n.d.
G^{12}		7.70	5.08	2.46	2.51	4.71	n.d.	n.d.
A^{13}		7.96	5.73	2.52	2.66	4.82	4.21	n.d.
A^{14}		7.92	5.94	2.31	2.66	4.75	4.22	n.d.
T^{15}	1.04	6.84	5.59	1.75	2.20	n.d.	n.d.	n.d.
C^{16}	5.32	7.24	5.38	1.85	2.19	n.d.	n.d.	n.d.
A^{17}		8.06	5.92	2.41	2.64	4.75	4.15	3.90, 3.85
T^{18}	1.20	6.92	5.25	1.69	2.02	n.d.	4.56	n.d.
A^{19}		7.90	5.79	2.41	2.60	4.75	4.13	3.81, 3.81
G^{20}		7.42	5.72	1.99	2.15	4.75	4.36	3.87, 3.87

Table 3S. Assignments of the exchangeable protons of AFB_1-FAPY modified and unmodified oligonucleotides. (5 °C)

Nucleotide	Protons	δ (Unmodified) (ppm)	δ (Modified) (ppm)	Δδ (ppm)
C^1	$N4H_b$	7.73	7.68	0.05
	$N4H_a$	7.22	7.24	0.02
T^2	N3H	13.6	13.6	0.00
A^3	$N2H_a$	6.38	6.41	0.03
	$N2H_b$	7.58	7.67	0.09
T^4	N3H	13.6	13.2	-0.40
$G^5/^{FAPY}G^5$	N1H	12.2	12.1	-0.04
A^6	$N2H_a$	5.85	6.55	0.70
	$N2H_b$	7.56	7.68	0.12
T^7	N3H	13.7	13.81	0.14
T^8	N3H	13.8	13.8	0.00
C^9	$N4H_a$	7.05	7.07	0.02
	$N4H_b$	8.41	7.98	-0.42
G^{12}	N1H	12.6	12.6	-0.02
A^{13}	$N2H_a$	5.92	5.96	0.04
	$N2H_b$	7.56	7.67	0.11
A^{14}	$N2H_a$	5.99	6.00	0.01
	$N2H_b$	7.58	7.65	0.07
T^{15}	N3H	13.4	13.3	-0.14
C^{16}	$N4H_a$	6.66	6.83	0.17
	$N4H_b$	8.22	8.23	0.01
A^{17}	$N2H_a$	6.21	6.75	0.54
	$N2H_b$	7.60	7.56	-0.04
T^{18}	N3H	13.4	13.3	-0.10
A^{19}	$N2H_a$	6.36	6.42	0.06
	$N2H_b$	7.72	7.71	-0.01
G^{20}	N1H	12.6	12.6	0.0

Table 4S. Partial charges for the atoms of the AFB_1-FAPY lesion $^{FAPY}G^5$.

	Atom Name	Atom Type[1]	Partial Charge[2]
1	P	P	1.076
2	O1P	O2	-0.514
3	O2P	O2	-0.45
4	O5'	OS	-0.362
5	C5'	C2	0.16
6	H5'	H	0.009
7	H5''	H	-0.001
8	C4'	CH	0.067
9	H4'	H	0.048
10	O4'	OS	-0.347
11	C1'	CH	0.273
12	H1'	H	0.058
13	N9	NS	-0.206
14	HN9	H	0.156
15	C4	CB	0.111
16	N3	NC	-0.324
17	C2	CA	0.382
18	N2	N2	-0.359
19	H21	H2	0.236
20	H22	H2	0.208
21	N1	NA	-0.379
22	H1	H	0.203
23	C6	C	0.436
24	O6	O	-0.328
25	C5	CB	-0.252
26	N7	NT	-0.179
27	C8	CO	0.046
28	O8	OA	-0.53
29	H8	H	0.201

30	C8A	CTA	0.191
31	H8A	H	0.051
32	C9	CTA	0.053
33	H9	H	0.083
34	O9	OH	-0.288

	Atom Name	Atom Type	Partial Charge
37	H9A	H	0.074
38	C9B	CBA	-0.255
39	O7	OJ	-0.253
40	C6A	CTA	0.221
41	H6A	H	0.056
42	O6A	OJ	-0.212
43	C5A	CBA	0.219
44	C5B	CAA	-0.139
45	H5B	H	-0.089
46	C4B	CBA	0.111
47	O4	OSA	-0.305
48	CM	CTA	0.201
49	HM1	H	0.022
50	HM2	H	0.021
51	HM3	H	0.022
52	C4A	CBA	-0.251
53	C10A	CBA	0.273
54	O10	OJ	-0.261
55	C11	CO	0.421
56	O11	OA	-0.26
57	C11A	CBA	-0.332
58	C3A	CBA	0.176
59	C3	CTA	-0.005
60	H31	H	0.049
61	H32	H	0.021
62	C2A	CTA	-0.083

63	H21	H	0.025
64	H22	H	0.017
65	C1	CO	0.311
66	O1	OA	-0.263
67	C2'	CH	0.115
68	H2''	H	0.035
69	O2'	OH	-0.4
70	H2'	HO	0.25
71	C3'	CH	0.219
72	H3'	H	0.007
73	O3'	OS	-0.79

[1]Atom types for $^{FAPY}G^{5}$ were assigned using the standard atom type in the CHARMM (v.2.2) force field.

[2]Partial charges were obtained from MOPAC calculation with a total charge of -1 assigned for the nucleotide.

[3]Atoms which were defined within a group in the residue topology file are separated by space.

Figure 1S. The atom names defined for the atoms of the $^{FAPY}G^{5}$ nucleotide.

Figure 2S. Stereoviews of the starting structures for the MD simulation/annealing structural refinement (A) iniB was generated from B-form DNA, (B) iniA was generated from A-form DNA.

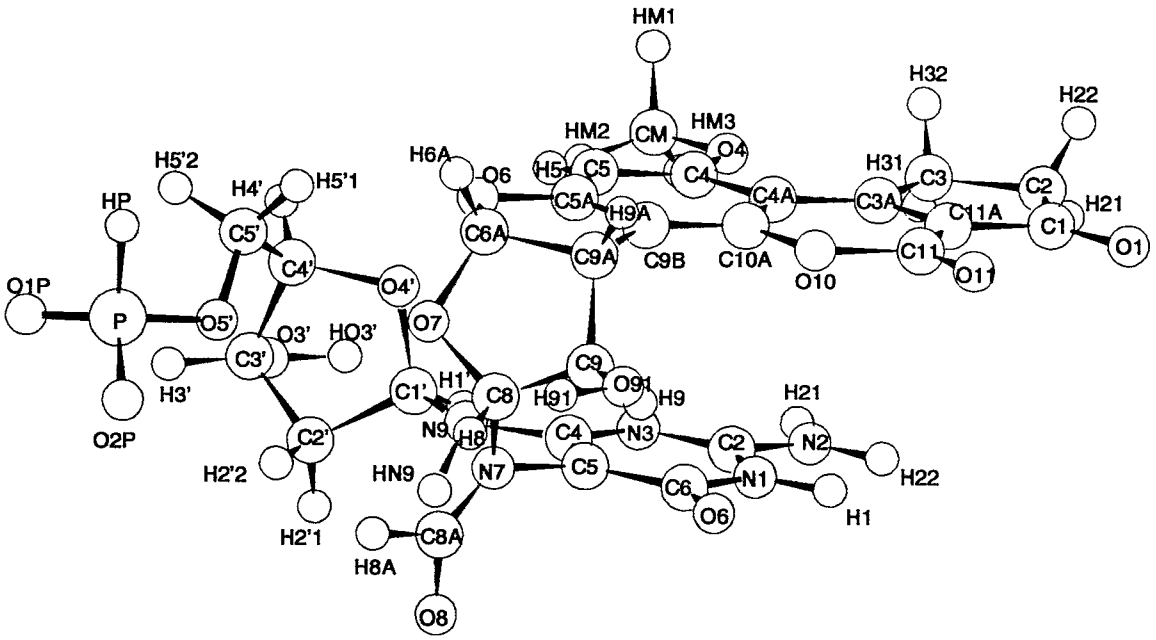
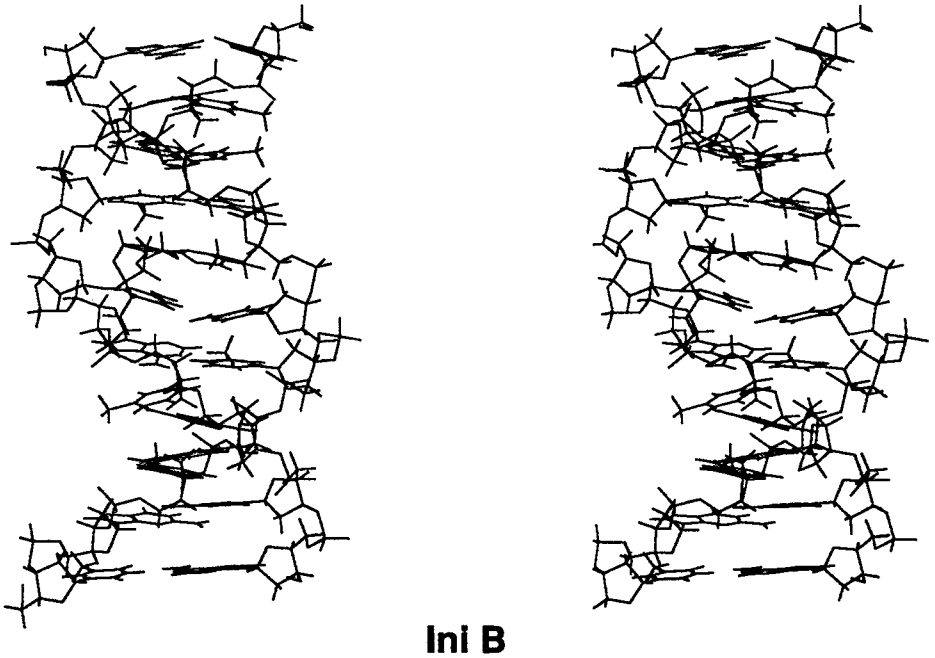

HM1
H32
H22
HM2
CM
HM3
H5'2
H6A
O6
H5
C5
C4
O4
H31
C3
C2
HP
H4'
H5'1
C5'
C5A
C4A
C3A
C11A
H21
C1
H9A
C6A
C9A
C9B
C10A
C11
O1
O1P
C4'
O4'
O10
O11
P
O5'
O3'
HO3'
O7
C9
H3'
C3'
C1'
H1'
C8
H91
O91
H9
H21
O2P
C2'
N9
H8'
C4
N3
C2
N2
H2'2
HN9
N7
C5
C6
N1
H22
O6
H1
H2'1
C8A
H8A
O8

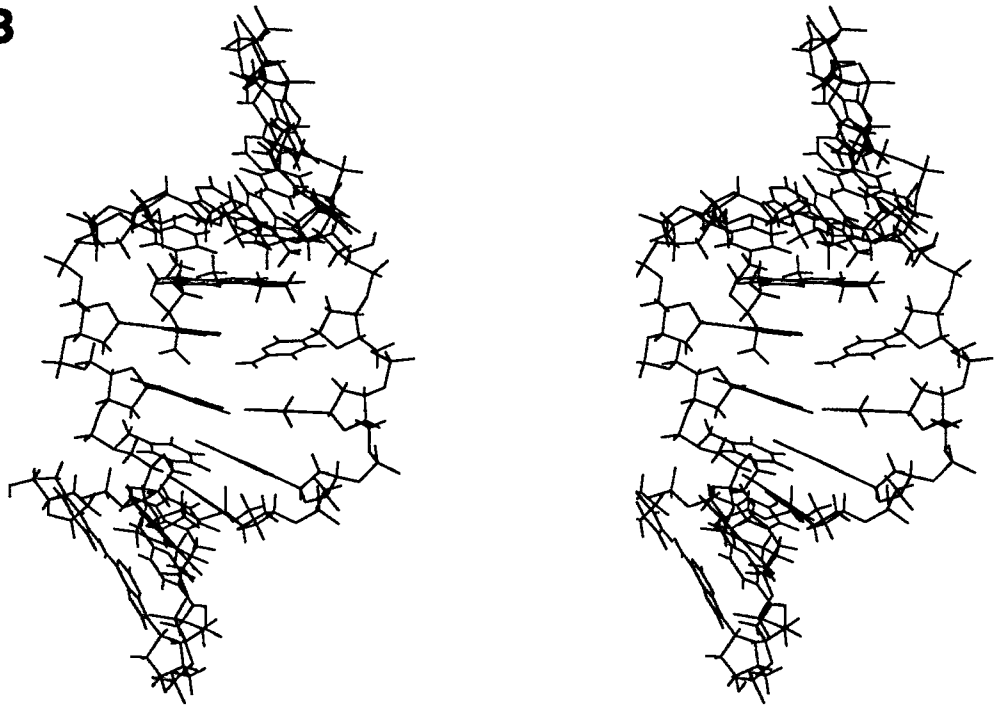
Figure 15

A



Ini B

B



Ini A

Figure 25