Articles

A Molecular Model for DNA Cross-Linking by the Antitumor Agent Azinomycin B

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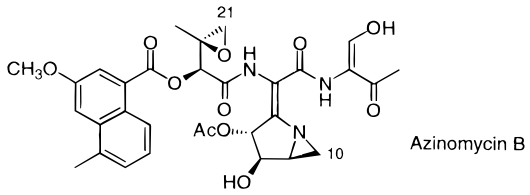
A computational model for the covalent interstrand DNA cross-linking of the antitumor agent azinomycin B is reported and is based on Monte Carlo simulations of the four possible monoalkylation species and an examination of the low energy conformations of the cross-linked agent. The model was developed using a suitably modified version of the AMBER* force field with the experimentally determined triplet DNA target sequence 5′-d(GCT)-3′ in both the native B-form and containing a preformed intercalation site.

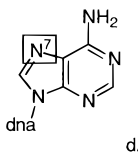
Introduction

Azinomycin B is an antitumor agent isolated from cultures of *Streptomyces griseofuscus*.[1] Azinomycin B and the related agent azinomycin A exhibit potent in vitro cytotoxic activity and effective in vivo antitumor activity against P388 leukemia in mice.[2] Biological evaluation of these agents has been hampered by chemical instability and poor availability from natural sources.[3]

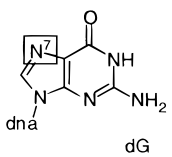


Azinomycin B

The presence of electrophilic epoxide and aziridine rings in the agents suggests that the azinomycins alkylate and cross-link DNA[4] via the C21 and C10 carbons. Studies on azinomycin/DNA interactions[5,6] have concluded that cross-linking occurs within the major groove via the N7 position of two purine bases. This unprecedented molecular mechanism of action and effective antitumor activity make the azinomycins particularly attractive targets for synthetic[7,8] and mechanistic efforts;[4,5] biological evaluation has been limited to the original report.[2]



dA



dG

We recently developed new AMBER* force field parameters and described Monte Carlo conformational searching of the natural agents.[9] In these studies we found a large number of populated conformers, and we developed a filtering protocol based on a distance and vector analysis of the cross-linking site on DNA to analyze low energy conformers. We concluded that azinomycin A and B were preorganized for DNA cross-linking.[9]

Understanding the interactions of azinomycin B with DNA by computational protocols is particularly challenging because of the conformationally mobile nature of the molecule,[9] compared with more rigid agents such as mitomycin C[10] or CC-1065.[11] In the present studies, we have examined the noncovalent association, monoalkylation, and interstrand DNA cross-linking by azinomycin B using an *unbiased* Monte Carlo simulation of the agents bound to the putative DNA target sequence 5′-d(GCT)-3′.[5,6] We have examined the interaction of the agent with both a native B-DNA receptor and with a B-DNA receptor containing a preformed intercalation site. Our results provide a preliminary model for the interaction of this mechanistically and structurally unique antitumor agent with duplex DNA that is consistent with experimental sequence selectivity.[5,6]

In our computational simulations of the interactions of azinomycin B with duplex DNA, the following schematic was considered (Scheme 1). The initial noncovalent association of the agent with DNA can occur by two alternative pathways: (1) simple association of the agent with the surface of the major groove; (2) association of the agent with the major groove accompanied by intercalation. Once this association occurs, two covalent bonds are formed in a sequential fashion between the electrophilic agent and nucleophilic DNA: monoalkylation followed by subsequent interstrand cross-link formation. Each of these steps may or may not be accompanied by intercalation. Herein, we examine both pathways in detail, focusing on the conforma-

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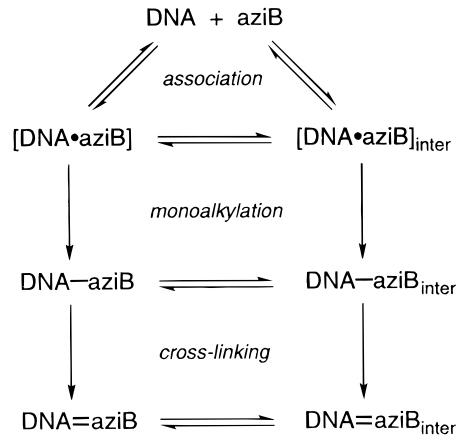
Scheme 1

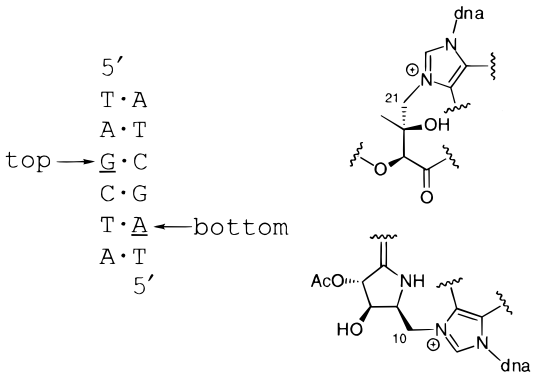


tional preferences of the agent when bound to DNA with respect to distance and geometric requirements for covalent bond formation between the electrophilic epoxide C21 and aziridine C10 carbons and the nucleophilic purine N7 atoms of the DNA.

Results and Discussion

Nonintercalated Model. Analysis of the noncovalent interactions of azinomycin B with duplex DNA was performed with both intercalative and nonintercalative binding modes[12] in order to understand the effect of preorganization of the agent for monoalkylation and cross-link formation. Using the program GlobMOLINE[13] and the set of minimum energy conformers described previously,[9] with no intercalative binding site, we could not find a structure that we judged competent to proceed to monoalkylation. This algorithm showed no discernible recognition between the agent and DNA.

There are four monoalkylation intermediates of the purine N7 atoms in the target sequence that must be considered: two aziridine C10 monoalkylation products and the two epoxide C21 monoalkylation products for the indicated 5′-disposed dA and dG bases.[14] We also examined the most plausible cross-linked product, where the aziridine C10 is bonded to the bottom dA-N7[14] as proposed by Saito et al.[5] In these simulations, we kept the DNA atoms fixed and examined the conformational variability of the azinomycin B fragment.



The DNA coordinates[15] were generated using the program MacroModel (v. 5.5)[16] in the B-conformation,[17] and Na^+ counterions were positioned 2.5 Å from each anionic oxygen of the phosphates. DNA and sodium atoms were fixed with force constants of 24 kcal/mol.

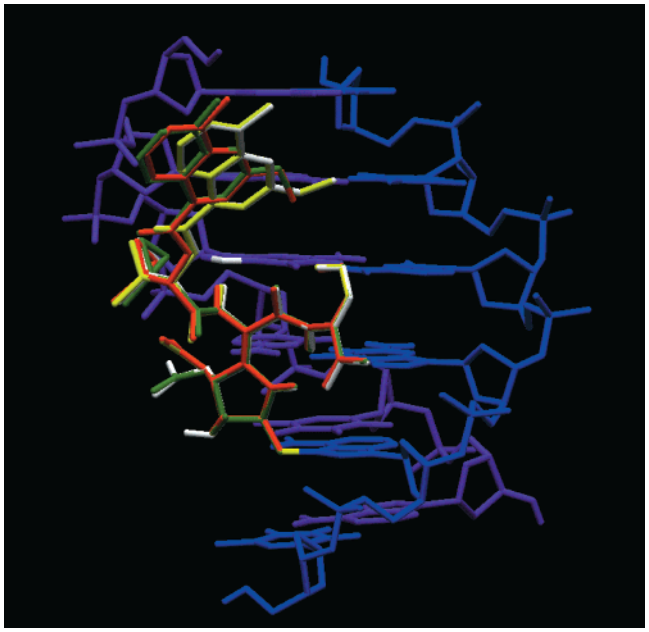


Figure 1. Molecular graphics representation of the four lowest energy MAB conformers. Coloring: DNA "top" – purple; DNA "bottom" – blue; "top" dG-N7 atom – white; agents (increasing energy) – yellow, white, green, red.

Table 1. Data for MAB Simulation

conf #	N7–C21 (Å)	N7–C–O (deg)	rel energy (kcal/mol)	Boltzman (%)
1	3.37	133.7	0.00	36.6
2	3.27	139.2	0.21	25.5
3	3.22	132.7	0.67	11.9
4	3.12	132.0	1.07	6.0
5	4.15	22.8	1.26	4.4
6	3.41	134.3	1.38	3.6
7	3.31	138.8	1.48	3.1
8	3.17	153.1	2.03	1.2
9	3.21	136.9	2.03	1.2
10	3.22	133.5	2.15	1.0

Starting with the minimum energy conformer of azinomycin B obtained in earlier studies,[9] the four starting monoalkylation intermediates were generated by the following methods: (1) energy minimization of the ring-opened epoxide and aziridine compounds; (2) attachment of the agent to the purine N7 by superpositioning C10 or C21 of the agents onto the carbon of an N7-methylpurine; (3) deletion of the methyl group; and (4) connection of the agent to the DNA.

Each of the four starting structures were subjected to a Monte Carlo simulation with 14 rotatable bonds on the azinomycin fragment including the purine N7–agent bond. Ten thousand conformations were generated and minimized[18] with our modified AMBER* force field[9] (≤3000 iterations) in vacuo ($\epsilon = 1$) and again with the GB/SA water model (≤5000 iterations).[19] Convergence was judged complete if the global minimum was found more than one time and remained unchanged during the latter portion of the calculation. Conformations >12 kcal/mol above the minimum were discarded in the in vacuo search.

The results of these simulations delineated a clear sequence dependence for the subsequent cross-link formation. Only the dA-aziridine monoalkylation simulation (MAB)[14] gave populated conformations that were geometrically competent to proceed to cross-linked

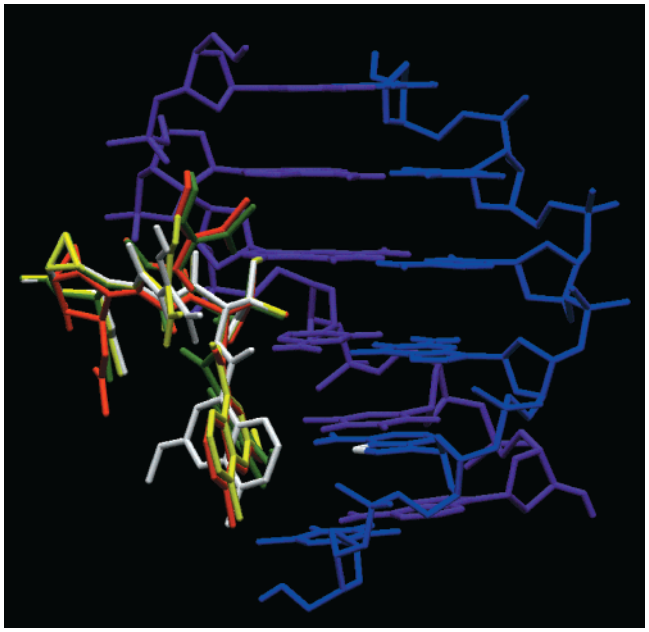


Figure 2. Molecular graphics representation of the four lowest energy MET conformers. Coloring: DNA "top" − purple; DNA "bottom" − blue; "bottom" dA-N7 atom − white; agents (increasing energy) − yellow, white, green, red.

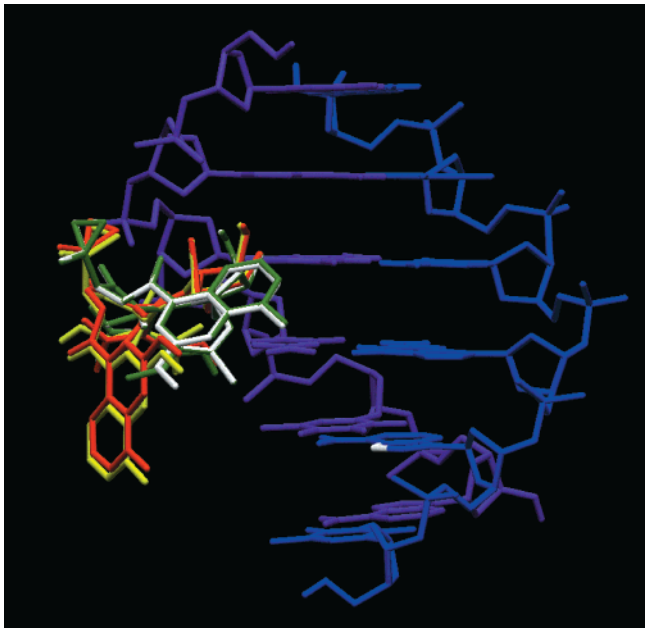


Figure 3. Molecular graphics representation of the four lowest energy MAT conformers. Coloring: DNA "top" − purple; DNA "bottom" − blue; "bottom" dA-N7 atom − white; agents (increasing energy) − yellow, white, green, red.

Table 2. Data for MET Simulation

conf #	N7−C10 (Å)	N7−C−N (deg)	Boltzman (%)
1	14.07	41.04	57.58
2	14.08	40.60	7.76
3	14.00	43.61	5.19
4	13.52	55.70	5.19
5	13.47	57.06	5.19
6	13.68	54.83	5.19
7	14.03	43.86	3.48
8	14.05	42.59	3.48
9	13.48	56.32	2.33
10	14.02	43.65	1.56

Table 3. Data for MAT Simulation

conf #	N7−C21 (Å)	N7−C−O (deg)	Boltzman (%)
1	14.28	52.25	13.57
2	14.88	60.87	9.09
3	14.89	61.32	6.09
4	14.38	54.44	4.08
5	13.56	88.31	4.08
6	14.32	58.04	4.08
7	13.66	86.50	4.08
8	14.29	54.17	4.08
9	13.95	83.26	2.73
10	14.32	61.97	2.73
11	14.32	54.66	2.73
12	14.32	62.52	2.73
13	14.34	58.14	2.73
14	13.74	87.55	1.83
15	14.25	79.34	1.83

product (Figure 1). Of the 10 lowest energy conformers found in the MAB simulation, ≤2.15 kcal/mol above the global minimum (Table 1), nine had the epoxide C21 suitably oriented with respect to the dG N7 for the second alkylation event (i.e., N7−C21 < 3.5 Å and ∠N7−C21−O > 130°). Boltzman analysis showed that conformers effectively disposed for the second alkylation event represented at least 93% of the *total* population at 300 K.

Support for a specific ordering of the two alkylation events was provided upon examination of the other monoalkylation systems. In the MET[14] simulation, which should, in principle, lead to the same cross-link as the MAB simulation, we observed no conformations of the agent that were capable of undergoing cross-link formation (Figure 2). The minimum aziridine C10 to dA-N7 distance was 12.1 Å in a conformer that was 4.5 kcal/mol above the minimum. The average distance for all conformers was 13.7 Å. The relevant distances and angles are shown in Table 2.

Similarly, for the MAT[14] simulation there were *no* conformations found within 18.4 kcal/mol of the global minimum that had an epoxide C21 to dA-N7 distance of <11.5 Å (Figure 3). Data for the first 15 conformations are shown in Table 3.

Finally, in the MEB[14] simulation (Figure 4), the average aziridine C10 to dG-N7 distance among conformers within 5.0 kcal/mol of the global minimum was 13.0 Å. The minimum distance found was 4.6 Å, but this was in conformations that were 8.4, 10.0, and 11.2 kcal/mol above the minimum with unproductive N7−C10−N9 attack angles near 12°. The data for this simulation are shown in Table 4.

In short, it is clear from a comparison of the four simulations that only dA-N7 alkylation by the aziridine C10 (MAB) could afford a system capable of a subsequent cross-linking event. In these three simulations (MAT, MEB, MET), very often the primary contacts between the agent and DNA were electrostatic interactions with the phosphate diester backbone. There were few hydrogen bonding contacts between the agents and DNA bases. Qualitatively, it appeared that the agent did not fit well within the major groove in these orientations. In contrast, the low energy conformers from the MAB simulation capable of cross-link formation formed a common series of hydrogen bonds between the agent backbone and the DNA bases.

We examined the conformational profile of the cross-linked species AB-ET[14] generated from the low energy MAB[14] conformer by connection of the dG-N7 to the

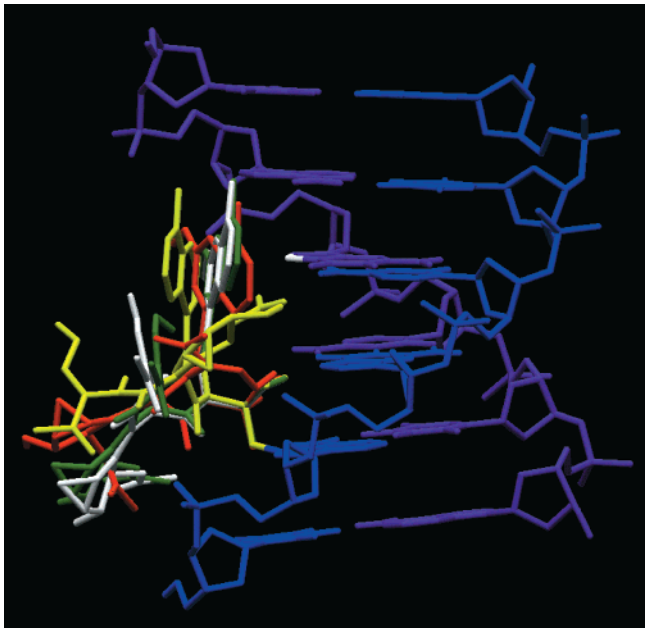


Figure 4. Molecular graphics representation of the four lowest energy MEB conformers. Coloring: DNA "top" – purple; DNA "bottom" – blue; "top" dA-N7 atom – white; agents (increasing energy) – yellow, white, green, red.

Table 4. Data for MEB Simulation

conf #	N7–C10 (Å)	N7–C–N (deg)	Boltzman (%)
1	11.81	54.72	43.37
2	13.86	50.57	43.37
3	13.65	55.86	3.91
4	12.84	70.87	2.62
5	12.98	65.60	2.62
6	11.39	64.47	1.17

epoxide C21 followed by energy minimization. This structure was subjected to a second Monte Carlo simulation using 17 rotatable bonds in the agent including both agent-N7 bonds. The central amide bond of the agent was selected as the closure bond. Ten thousand conformations were generated and minimized in vacuo and subsequently with the GB/SA water model. In this simulation, we observed a very close correspondence between the cross-linking competent conformers from the MAB monoalkylation simulation and the low energy conformers of the cross-linked species (Figure 5), indicating that minimal structural reorganization of the agent is required for formation of the second covalent bond.

With some agents, cross-linking distorts the DNA significantly from a B-form helix (e.g., cisplatin[20] or nitrogen mustard[21]). In these cases, there is a poor correspondence between the geometry of the agent (i.e., the "reach" or distance between the two electrophilic sites) and the nucleic acid binding site, and for cross-linking to occur the DNA must undergo significant distortion. With azinomycin B, however, we demonstrated by a distance and vector analysis that the DNA cross-linking site corresponds well with the geometry of energetically populated conformers of the agent,[9] and evidence from the present study suggests that a minimal distortion of the B-DNA duplex is *necessary* for cross-linking by this agent to occur.

To confirm this conclusion, we compared the minimum energy conformation of the cross-link AB-ET[14]

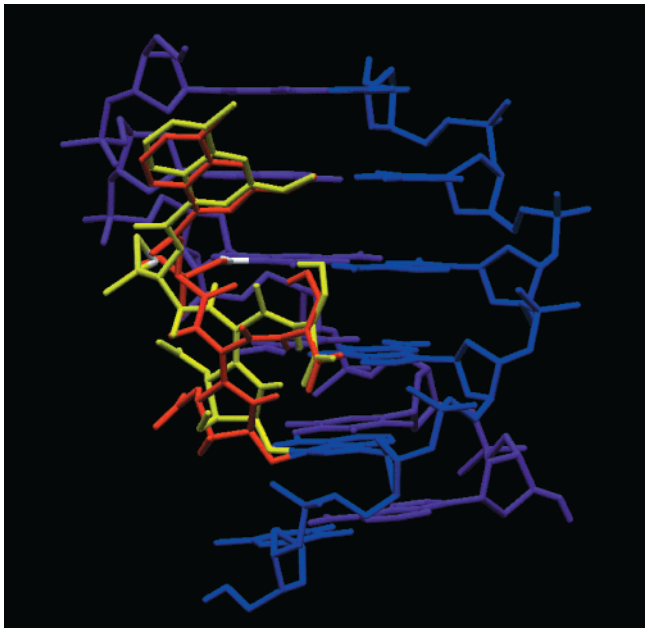
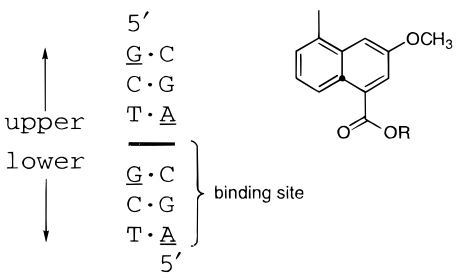


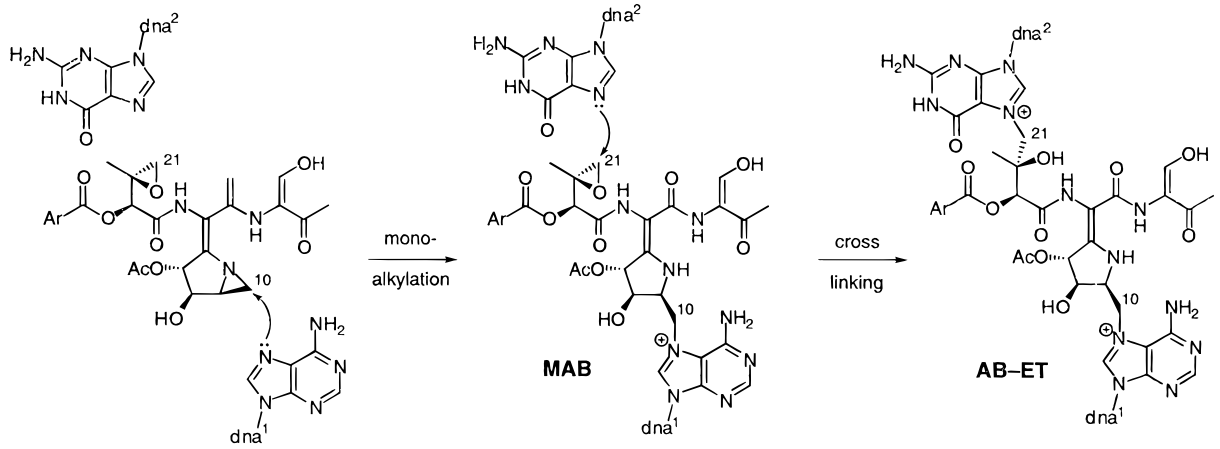
Figure 5. Molecular graphics representation of the MAB low energy conformer and the low energy conformation of the proposed covalently cross-linked duplex. Coloring: DNA "top" – purple; DNA "bottom" – blue; aziridine monoalkylation product (MAB) – yellow; cross-linked agent (AB-ET) – red; purine-N7 and epoxide C21 atoms – white.

obtained using the rigid DNA model with the Monte Carlo energy minimum of AB-ET obtained *without* constraints on the DNA atoms (data not shown). The dA-N7 to dG-N7 distance changed only slightly, from 8.9 Å in the rigid DNA minimum to 9.1 Å in the relaxed DNA minimum (1.3 Å RMS deviation), well within the noise of base movements seen in molecular dynamics trajectories. It appears that azinomycin B is able to effectively alkylate B-form DNA with minimal structural variation of either the agent or the DNA,[22] in contrast to agents such as the nitrogen mustards.

Intercalative Model. Using an intercalative binding site extracted from an X-ray crystal structure,[23] we built the following DNA receptor that allowed the drug to interact with two distinct upper and lower binding sites.[24] Thus, all four orientations of the drug could be evaluated in one Monte Carlo simulation. The specified naphthalene carbon was constrained at a distance of 3.5 Å from the adjacent dG base with a force constant of 20 kJ/mol.[25] This constraint was considered weak enough to allow the naphthalene to make modest movements, but strong enough to keep it intercalated. Constraining only one carbon of the naphthalene allowed the aromatic ring to flip by 180°, thereby obviating the need to perform two simulations to examine both orientations of the intercalated aromatic system.



Scheme 2



Monte Carlo protocols were as before with the DNA atoms fixed. The in vacuo simulation was resubmitted for minimization with GB/SA water treatment *without* the naphthalene constraint. We examined the first 29 conformations that were within 5 kcal/mol of the global minimum. Applying a cross-link criterion of nucleophile−electrophile distance of ≤4.50 Å, there were five accepted conformations at 1.91, 2.15, 4.54, 4.78, and 5.00 kcal/mol above the global minimum wherein both the aziridine and epoxide were suitably positioned for cross-link formation.[26] Notably, all configure cross-linking within the lower site, where the aziridine alkylates the bottom dA-N7 and the epoxide alkylates the top dG-N7. Among these conformations, the fourth possesses the lowest N7−C10 and C21 distances, both equal to 3.16 Å. Also the attack orientations are compatible with the cross-link and are for the aziridine and the epoxide opening, respectively, 158° and 115°. On the upper site there are no conformations compatible with the complementary mechanism of cross-link, i.e., aziridine top (dG-N7) and epoxide bottom (dA-N7). Our conclusion is that an intercalative association is only compatible with cross-link formation with the aziridine bottom (dA-N7) and epoxide top (dG-N7) in the lower binding site, but that this mode of binding requires further investigation, both computationally and experimentally.

Conclusions

In these studies, we have developed a realistic model of the details of the covalent interaction of this structurally and mechanistically unique antitumor agent with duplex DNA. Our preliminary results are consistent with experimentally determined sequence selectivities[5,6] and are based on an unbiased and rigorous conformational analysis of all possible covalent and noncovalent intermediates formed between the agent and DNA. We have predicted the same sequence selectivity regardless of the role of the naphthalene.[13] We have provided evidence that DNA cross-linking by azinomycin B occurs through an initial alkylation of the adenosine by the aziridine C10 followed by alkylation of the guanosine by the epoxide C21 to effect covalent cross-link formation (Scheme 2), consistent with Saito's proposal.[5]

A detailed computational study of the DNA cross-linking by the azinomycins will include a correlation with the experimentally determined sequence dependencies, an examination of structural reorganization of the DNA upon cross-link formation, and a determination of the roles of various structural elements in the initial DNA·agent binding event.

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(14) MAB = *m*onoalkylation *a*ziridine *b*ottom; MAT = *m*onoalkylation *a*ziridine *t*op; MEB = *m*onoalkylation *e*poxide *b*ottom; MET = *m*onoalkylation *e*poxide *t*op; AB-ET = *a*ziridine *b*ottom *e*poxide *t*op cross-link.
(15) As proposed by Arnott and implemented in MacroModel. See: Arnott, S.; Campbell-Smith, P.; Chandresekharan, P. *CRC Handbook of Biochemistry*; CRC Press: Boca Raton, FL, 1976; Vol. 2.
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(17) Saito and co-workers used a short 6-mer of duplex DNA for their experimental studies.[5] This raises the nontrivial issue of the relevance of the present modeling work using canonical B-DNA to experimental results using a short and probably noncanonical sequence. While we cannot address the conformation of the 6-mer used by Saito and co-workers, in related experimental studies we have confirmed the regioselectivity proposed in the our present modeling work under conditions where a 15-mer DNA target is expected exists in the B-form (Coleman, R. S.; Perez, R. J. Unpublished work).
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(24) It is not possible for the drug to intercalate within the base triplet that undergoes cross-linking because the maximum C21 to C10 distance of azinomycin B is ≈ 9.5 Å compared to the N7 to N7 distance of 11.6 Å with an intercalation site placed in a d(GCT) B-form sequence.
(25) In the vast majority of X-ray crystal structures of molecules intercalated in duplex DNA, the center of the intercalating agent and the center of the upper and lower base pairs (measured from the four glycosidic nitrogens) are nearly coincident. The fixed distance approximated this coincidence.
(26) It is geometrically impossible for the naphthalene to be intercalated and the epoxide to alkylate the distal purine base.

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