Studies on DNA-cleaving agents: Computer modeling analysis of the mechanism of activation and cleavage of dynemicin–oligonucleotide complexes

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ABSTRACT Dynemicin A is a recently identified antitumor antibiotic. Upon activation, dynemicin is reported to cause double-stranded cleavage of DNA, putatively through the intermediary of a diradical. Computer modeling of this activation and cleavage process is described herein as part of an effort to establish a structural hypothesis for this mechanistic sequence and for the design of simple analogues. Intercalation complexes of duplex dodecamers [d(CGCGAATTCGCG)]2 and [d(GC)]2 with both enantiomers of dynemicin and of all related mechanistic intermediates are evaluated. Examination of these structures shows that cycloaromatization of dynemicin to a diradical intermediate results in the rotation of the diradical-forming subunit with respect to the intercalation plane that is of an opposite sense for the two dynemicin enantiomers. In addition, the activation of the (2S) enantiomer of dynemicin occurs by a less restricted approach trajectory than the corresponding (2R) enantiomer. In all complexes, the 5′-3′ strand is at least 1 Å closer than the 3′-5′ strand to the diyl intermediate. As a result, complexes are produced in which the diyl moiety is aligned along [(2S)] or [(2R)] the minor groove, leading to different predictions for the selectivity of radical-initiated, oxidative lesion of DNA. Molecular dynamics simulations are found to support these predictions, including the 3-base-pair offset cleavage reported for dynemicin.

The cleavage of DNA is a key process in the transfer of genetic information, the mode of action of certain chemotherapeutic agents, and the function of reagents designed for DNA modification and structure determination. DNA cleavage can be effected with a variety of agents ranging from the simple hydroxyl radical to relatively complex restriction enzymes. Within the past 5 years, the antimutator antibiotics calicheamicin (1), esperamycin (2), and neocarzinostatin (3) have emerged as new structural and mechanistic class of DNA-cleaving agents that are proposed to operate through the inductive generation of an arenyl or indenyl diradical. The most recently identified member of this class is dynemicin A (structure I in Scheme I), a compound that exhibits potent cytotoxicity and in vivo antitumor activity (4, 5). Dynemicin has been shown to interact with the minor groove of DNA and, upon activation, to cause double-strand breaks 3 base pairs (bp) apart (6). Examination of the structure of dynemicin suggests that it could be activated for DNA cleavage through reduction of its anthraquinone subunit, resulting in heterolysis of the adjacent epoxide ring. Addition of a nucleophile to, or protonation of, the resultant anthraquinone methide (structure 2 in Scheme I) would provide an activated derivative 3 which, in the absence of the constraints imposed by the original epoxide ring, would undergo facile cycloaromatization to diradical 4 (Scheme I). (Heats of formation were determined by using the AMPAC program (QCPE no. 506, version 2.1) with the AM1 Hamiltonian (7).) Abstraction of proximate deoxyribosyl hydrogens by this diradical would initiate oxidative cleavage on opposing DNA strands. Conversion of diradical 4 to the alternative ene–diyne structure 7 is not observed.

Semiempirical (8) and molecular mechanics (9) studies on dynemicin itself have provided valuable information in support of the above mechanism. Thus far, however, computational methods have not been used to evaluate the role of DNA in the mode of action of dynemicin, although they have been applied to calicheamicin (10) and neocarzinostatin (11), yielding models that are consistent with known DNA cleavage patterns. We describe herein computer modeling studies designed to delineate at the molecular level the interaction of dynemicin and dynemicin-derived intermediates with oligonucleotides selected to emulate native DNA. These studies address several fundamental issues that are crucial to the development of a structural hypothesis for the mode of action of dynemicin and its analogues, including (i) the mechanistic fate of the two possible enantiomers of dynemicin [structures 1-{2R} or 1-{2S}], a point of much interest since the absolute stereochemistry of dynemicin is as yet unknown, (ii) the effect of nucleophile size and approach trajectory in the activation step and the dynamics of this activation process, (iii) the influence of oligonucleotide sequence and length on dynemicin intercalation and activation, and (iv) the relationship of intercalation sites to cleavage sites. The answers to these questions provide a structural basis for evaluating mechanistic proposals, for predicting DNA cleavage patterns, and for designing new cleaving agents based on the dynemicin lead.

METHODS

A DNA octamer corresponding to [d(CGCGAATTCGCG)]2 and dodecamers corresponding to [d(CGCGAATTCGCG)]2 and [d(GC)]2 were constructed in B-DNA form by using the program MACROMODEL (versions 2.0 and 3.0; W. C. Still, Columbia University) running on a MicroVax 3900 and Evans and Sutherland PS340 systems. All sequences were minimized to a gradient of <0.100 kJ/mol per Å under the AMBER force field (12) before intercalation experiments were begun. Both enantiomers corresponding to dynemicin A 1, the putative quinone methide intermediate 2, the proposed quinone methide addition product 3, and a surrogate for the cyclized, diradical intermediate 5 were minimized to a gradient of <0.200 kJ/mol per Å by using the MM2 force field (13, 14). A surrogate for diradical intermediate 4 was necessary because cyanomolecular mechanics force fields are not parameterized for diradicals; the pyrazine ring was chosen as a surrogate because of its geometric similarity to the putative diradical species. As the current parameter set available in

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**RESULTS**

Because of the absence of DNA cleavage data at the outset of this study, G+C- and A+T-rich oligonucleotide sequences were selected to explore two generic intercalation sites. Sequence selection around these sites was guided by symmetry considerations (to simplify calculations) and/or by the availability of solid state (18) or solution phase (19–24) structural information (for calibration). Sequence length was initially set at 8 bp to minimize computational time. However, an early finding of this study was that the intercalation complexes of the resultant duplex octamers exhibit disrupted base pairing at terminal residues when full, rather than substructure, minimizations were conducted. Since this modeling suggested, in agreement with solution studies of DNA intercalation complexes, that the effects of intercalation are transmitted more than four residues in both directions from the site of intercalation, the oligonucleotide length was extended to duplex dodecamers. The resultant duplex structure for uncomplexed [d(CGCGAATTCGCG)], was found to differ only slightly from the x-ray crystal structure (18), the latter being slightly more tightly wound as a result of crystal-packing forces. Intercalation complexes of both duplex dode-
cameras produced a DNA model that displayed normal end
group base pairing.

Since the absolute stereochemistry of dynemicin has not
been established, intercalation complexes were constructed of
the aforementioned duplex dodecamers with both possible
enantiomers of dynemicin and of the resultant quinone meth-
tide, cyclization precursor, and pyrazine surrogate for the
dirdical. All minimized duplexes were found to be in the
right-handed, Watson–Crick B form, with most of the sugar
ring puckers C2'-endo. The overall structure of the oligonu-
cleotides in the complexes was little changed from the uncom-
plexed form. Separation of the base pairs immediately above
and below the intercalation site increased from 3.4 Å to
6.4–6.6 Å upon intercalation of dynemicin, and Watson–Crick
base pairing remained intact. The intercalation site was wedge-
shaped, similar to that observed for a nogalamycin–
oligonucleotide complex (25) and a daunomycin–oligonucleo-
tide complex (26). Base pairs directly adjacent to the interca-
lation site exhibited a slight propellor twist and were buckled.
The anthraquinone moiety was puckered and not planar.
Although this puckering is not observed in the daunomycin
complex, it is seen in nogalamycin, which, like dynemicin, has
an aliphatic ring rigidly attached to the intercalator subunit
(25).

Two general energy minima were found for the complexes,
one with the anthraquinone intercalated more deeply and the
ene–diyne moiety closer to the double-helical axis than in the
other. The proposed explanation for this is that H5' and/or
H4' of base 8 cause unfavorable Van der Waals interactions
when they are in the same plane as the ene–diyne (or diradical
surrogate) portion. This results in a local minimum when the
ene–diyne/diradical surrogate is inserted beyond these hy-
drogens. Although the size of the barrier between these two
minima is unknown, experimental data available for the
structurally related daunomycin–oligonucleotide complex, in
which the amino sugar lies on the floor of the minor groove
and the D ring protrudes into solution from the major groove,
suggests that deeply intercalated species are energetically
accessible. This also suggests that intercalation depth may be
an important structural parameter in the design of analogues
for abstraction of specific hydrogens.

Comparison of these complexes provides an understanding
of the structural changes that attend the activation and
cyclization steps, including the differing roles of the two
possible dynemicin enantiomers. Specifically, for the
conversion of the (2S)-dynemicin complex (Fig. 1 Left) to the

![Fig. 1. (2S)-dynemicin (Left) and (2R)-dynemicin (Right) in [d(CGCGAATTCGCG)]2.](image1)

![Fig. 2. (2S)-diyl surrogate (Left) and (2R)-diyl surrogate (Right) in [d(CGCGAATTCGCG)]2.](image2)

![Fig. 3. Corey–Pauling–Koltun models of the (2S)-diyl surrogate (Left) and (2R)-diyl surrogate (Right) in [d(CGCGAATTCGCG)]2. The closest hydrogen for each static complex is highlighted in red.](image3)
cumbrance from the 3’–5’ strand of the oligonucleotide. This trend is further supported by the relative stabilities of the activated complexes: in all cases, the (2S) complex is more stable than the (2R) complex, with energy differences ranging from 8.82 kcal/mol for nucleophiles similar to a methyl group in size to 14.72 kcal/mol for a tert-butyl group. For an activation process involving protonation of an anthraquinone methide, the product complexes are of comparable energy, suggesting that either could be accommodated in the minor groove. However, protonation of the (2R)-anthraquinone subunit would preferentially occur prior to association with DNA, since direct protonation of the anthraquinone subunit when complexed with DNA would be sterically disfavored. Alternatively, a relay proton transfer mechanism could be involved, in which case a small proton transfer agent (e.g., water) could associate with DNA prior to the intercalation of dynemicin and might remain present in the minor groove so as to allow direct contact with dynemicin.

An analysis of distances between abstractable hydrogens on the sugar–phosphate backbone and radical centers on 4 provides an indication of the manner in which cleavage sites are related to intercalation sites and suggests where the initial abstraction might occur. It was found that the 5’–3’ strand was, on average, greater than 1 Å closer to the “diyl” than the 3’–5’ strand in all of the static cases examined. For both (2S)-diyl complexes, the hydrogens on base 8 are closest. (The optimum transition-state geometry for abstraction of hydrogen from methane by methyl radical has been determined by ab initio methods; see ref. 27.)

Molecular dynamics simulations of the complexes of 5-(2R) and 5-(2S) with [d(CGCGAATTCGCG)]2 at 300 K confirm the above general observations on the static complexes and provide additional information with respect to the differing interaction of the two enantiomers with DNA. While base 7 is once again closest to the “diyl” moiety of 5-(2R) throughout the simulation, the contrasting handedness of 5-(2S) coupled with motion within the intercalation site causes base 8 to be closest in the 5-(2S)–DNA complex (Fig. 4). None of the bases of the 3’–5’ strand come within abstraction distance of the 5-(2R) “diyl” during the simulation, the closest being

![Fig. 4. Maximal (•), minimal (○), and average (+) N–H5’, N–H4’, N–H3’, and N–H1’ distances for 5 in [d(CGCGAATTCGCG)]2 from molecular dynamics simulations.](image)

![Fig. 5. Groove widths for [d(CGCGAATTCGCG)]2 and [d(GC)]2 complexes. 2S-1, 2R-1, 2S-5, and 2R-5, (2S) and (2R) enantiomers of structures 1 and 5 of Scheme I.](image)
base 18 at an approximate minimum distance of 4 Å. Bases 18 and 19 approach within 2 and 3.5 Å, respectively, of the 5-(2S)-“dicyl”.

Plots of groove widths measured for each dynemicin model intercalated into \([\text{dCGCGAATTCCGCG}]_2\) and for the free dodecamer are shown in Fig. 5. As expected from the “wrong-handed” twist observed on cyclization of 3-(2R), intercalation of 5-(2R) and its diyl model caused greater broadening of the minor groove than intercalation of 5-(2S). The larger groove widths observed for the (2R) enantiomer relative to the (2S) enantiomer in \([\text{dCGCGAATTCCGCG}]_2\) systems are not observed for the GC dodecamer \([\text{GC}]_2\) (Fig. 5). It has been observed (15) in x-ray diffraction studies on DNA fibers that the minor groove width of B-DNA increases with the proportion of G-C base pairs, and it is probable that the larger minor groove width of the GC dodecamer permits intercalation with less distortion.

**CONCLUSION**

Sixteen intercalation complexes of both possible enantiomers of dynemicin and of dynemicin-derived intermediates with duplex octamers and dodecamers were constructed and minimized. While the duplex octamer complexes suffered from disrupted base pairing at the termini, complexes with duplex dodecamers exhibited normal end group base-pairing. This suggests that duplex octamers may not be a reliable model for DNA in dynemicin-oligonucleotide complexes, and longer oligonucleotides should be used. Both enantiomers of dynemicin and putative precyclization intermediates yield intercalation complexes with similar orientations of the ene-diyne within the minor groove of DNA. Upon examination of the trajectory of attack of a nucleophile to the quinone methide intermediate, however, differences between the two enantiomers become apparent. The approach of a nucleophile to either enantiomer is hindered by the 3'-5' strand of DNA, and the effect is greater for the (2R) enantiomer. In addition to this kinetic effect on activation, it is also found that the activated (2S) complex is lower in energy than the (2R) complex for various nucleophiles. Cyclization to the diyl intermediate results in a rotation of the diyl moiety with respect to the intercalation plane, improving the alignment of the (2S) enantiomer within the minor groove, while causing the (2R) enantiomer to become aligned across the minor groove. Although it is not yet known whether dynemicin intercalates before or after activation occurs, the above evidence suggests that if intercalation occurs first, the (2S) enantiomer of dynemicin is better suited for nucleophile activation within the complex and also indicates that any nucleophile attacking at C8 of the quinone methide intermediate must be small.

In all complexes, the diyl is much closer to the 5'-3' strand of the oligonucleotide than to the 3'-5' strand. Even so, significant motion of the intermediate within the intercalation complex would be expected to occur, permitting abstraction from the 3'-5' strand. Molecular dynamics simulations designed to address this issue suggest that, although motion within the intercalation site brings the 5-(2S)-diyl surrogate within abstraction distance of both strands of DNA, abstraction from the 3'-5' strand would necessarily follow abstraction from the 5'-3' strand for the complex with 4-(2R).

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