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PREDICTED MODE OF INTERCALATION OF DOXORUBICIN WITH DINUCLEOTIDE DIMERS

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SUMMARY: Intermolecular molecular mechanics energy calculations have been carried out for doxorubicin interacting with two dinucleotide dimer sequences. The preferred mode of intercalation is in the minor groove with the anthraquinone ring of the drug nearly perpendicular to the base pairs for the (CpG) sequence having alternate C3' *endo*-C2' *endo* sugar ring puckering. The preferred intercalation conformation of the drug is nearly identical to the N-bromacetyldaunomycin crystal structure. This prediction is qualitatively consistent with the recently reported crystal structure of a d(CpGpCpGpCpG) dimer-daunomycin complex. For the other dinucleotide sequence, (TpC-ApG), minor groove intercalation is also preferred, but the drug conformation can be changed.

Detailed molecular models have been proposed for the intercalation of daunomycin and doxorubicin (see fig. 1) with DNA.[1-4] In these models the drug intercalates into the major groove of the DNA such that the long axis of the anthraquinone ring is nearly parallel to the long axes of the adjacent DNA base pairs. Proposed intercalation models differ from one another with respect to modes of intermolecular hydrogen bonding and formation of an electrostatic bond between the charged sugar amine of the drug and a phosphate of the DNA backbone.

We have modeled the intercalation of doxorubicin (I) with dinucleotide dimer sequences using molecular mechanics energy calculations.[5] Dimer sequence, type of sugar ring puckering, entry into the major and minor grooves of the dinucleotide dimer mini-helix, drug conformation, and charged state of the primary sugar amine group have been considered in our study. Our findings suggest that the preferred mode of intercalation is different from the proposed models.

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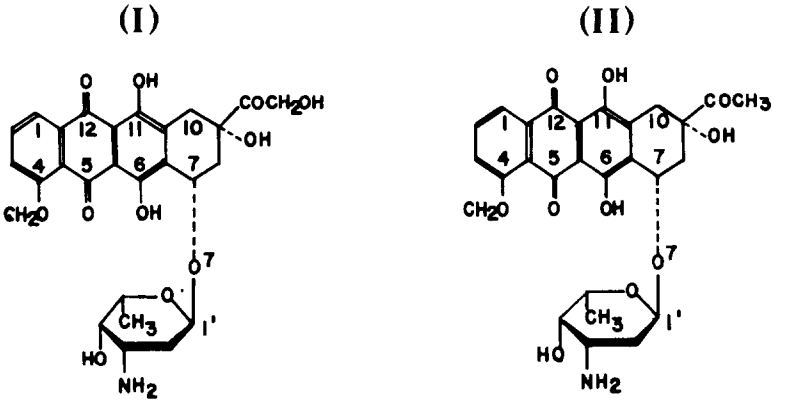


Fig. 1 Chemical structures of (I) doxorubicin, (II) daunomycin.

METHODS

A specialized version of the MASS (Molecular Assembly Software System) option in the CAMSEQ-II software system[6] was used to carryout the molecular mechanics calculations. The resident CAMSEQ-II potential energy functions were used with the residual charge densities computed using CNDO/2.[7] A total of 144 systematically selected intermolecular starting positions, involving both the major and minor grooves, were chosen in each drug-dinucleotide energy minimization. Two dinucleotide sequences,

$$\left\downarrow\left(\begin{matrix}C & - & G\\ P & & P\\ G & - & C\end{matrix}\right)\right\uparrow \quad \text{and} \quad \left\downarrow\left(\begin{matrix}T & - & A\\ P & & P\\ C & - & G\end{matrix}\right)\right\uparrow$$

, were considered in our study.

The dinucleotide dimer structure of the ethidium ion CpG crystal complex[8] and the most stable, unwound B-form-like dimer structure, found in our DNA unwinding calculations, [9] were each used for both sequences in the study. In the case of the calculated dimer structure, both C3' *endo*-C2' *endo* and C2' *endo*-C2' *endo* sugar ring puckerings were independently considered. The dinucleotide geometry was held fixed in each intermolecular energy minimization.

Three initial conformations of doxorubicin were used; the bromoacetyl crystal structure,[10] and the two conformations found to be intramolecular

energy minima.[11] The intercalation energy was minimized as a function of the doxorubicin (for both the neutral and charged form) degrees of conformational freedom and the six intermolecular degrees of freedom.

Table 1

Intercalation Properties of Doxorubicin with Two Dinucleotide Dimer Sequences

Dinucleotide		Doxorubicin		Intermolecular Energy[e]	
Sequence[a]	Position[b]	Conformation[c]	Form[d]	Complex	Isolated Dinucleotide
D1	minor	AM	NH_2	-124.08	-16.06
		AX		-118.38	-17.11
		AD		-124.16	-16.62
	major	AM	NH_2	-112.43	-16.06
		AX		-116.64	-17.11
		AD		-118.61	-16.62
D2	minor	AM	NH_2	-117.74	-15.80
		AX		-117.48	-16.96
		AD		-121.89	-16.46
	major	AM	NH_2	-114.73	-15.89
		AX		-103.78	-16.96
		AD		-104.27	-16.46
D3	minor	AM	NH_2	-123.87	-40.77
		AX		-127.99	-41.33
		AD		-132.60	-40.84
	major	AM		-123.05	-40.77
		AX		-104.73	-41.33
		AD		-105.99	-40.84
D3	minor	AM	NH_3^+	-109.20	-38.72
		AX		-107.91	-39.80
		AD		-113.98	-39.42
	major	AM		-111.66	-38.72
		AX		-105.31	-39.80
		AD		- 96.43	-39.42
r1	minor	AM	NH_2	- 73.62	-44.42
		AX		- 90.73	-45.46
		AD		- 61.21	-44.95
	major	AM		-101.02	-44.42
		AX		- 94.38	-45.46
		AD		- 94.49	-44.95

(a) D1-(TpC-ApG), C2' *endo*-C2' *endo*; D2-(TpC-ApG), C3' *endo* -C2' *endo*; D3-(CpG-GpC), C3' *endo* -C2' *endo*, r1-(i^5UpA·Et) from ref. 8.

(b) Refers to entry into the major or minor groove of the mini-helix of the dinucleotide dimer.

(c) AM - global intramolecular minimum energy conformation as reported in ref. 11.
AX - secondary intramolecular minimum energy conformation as reported in ref. 11.
AD - N-bromoacetyldaunomycin crystal conformation from ref. 10.

(d) Refers to sugar amine group being neutral or charged.

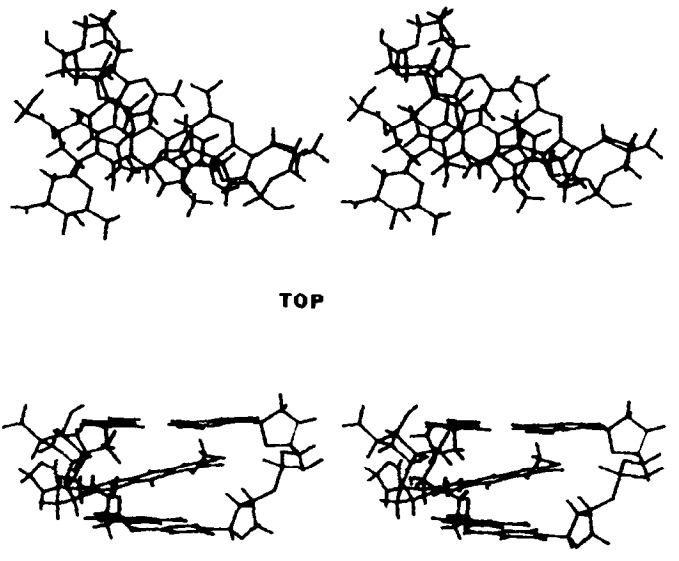
(e) Complex refers to the minimum energy intercalation structure, the energy units are kcals/mole.

RESULTS AND DISCUSSION

The minimum energy complexes, with respect to major and minor groove intercalation, for each dinucleotide dimer sequence and conformation, are reported in Table I. A consistent finding is that minor groove intercalation is preferred except for the (i^5 UpA·Et) dinucleotide dimer geometry. The energy preference for minor groove intercalation decreases for the ionized form of doxorubicin. Minor groove intercalation is characterized by the anthraquinone ring inserting between base-pairs with its long axis nearly perpendicular to the long axes of the base pairs. This mode of intercalation is qualitatively consistent with that observed for a d(CpGpCpGpCpG) dimer-daunomycin crystal complex by Rich and coworkers,[12] as well as some recent NMR studies of solution complexes of nucleic acid oligomeric dimers with daunomycin.[13,14] There is a dependence on the "angle" and "depth" of intercalation on base pair sequence. This is a consequence of minimizing the electro static potential between atoms in the two base pairs and those in the anthraquinone ring. The N-bromoacetyldaunomycin crystal conformation of the drug is preferred for minor groove intercalation with the (CpG) dimer structure. This conformational preference is lost for intercalation with (TpC-ApG). Also, mixed sugar ring puckering is not favored for the (TpC-ApG) sequence.

The predicted minor groove intercalation structure is stablized by complementary electrostatic interactions between the adjacent base pairs with the anthraquinone ring. A weak hydrogen bond/electrostatic interaction is realized between the sugar amine and a backbone phosphate group. The nine-position hydroxyls also have favorable electrostatic interactions with either phosphates or base-pair groups depending upon drug conformation and dinucleotide sequence.

The preferred modes of major and minor groove intercalation are shown in stereo in fig. 2 for (CpG). The mode of major groove intercalation is similar to proposed models in that the anthraquinone ring is nearly "parallel" to the base pairs. Proposed major groove intercalation models[1-4]



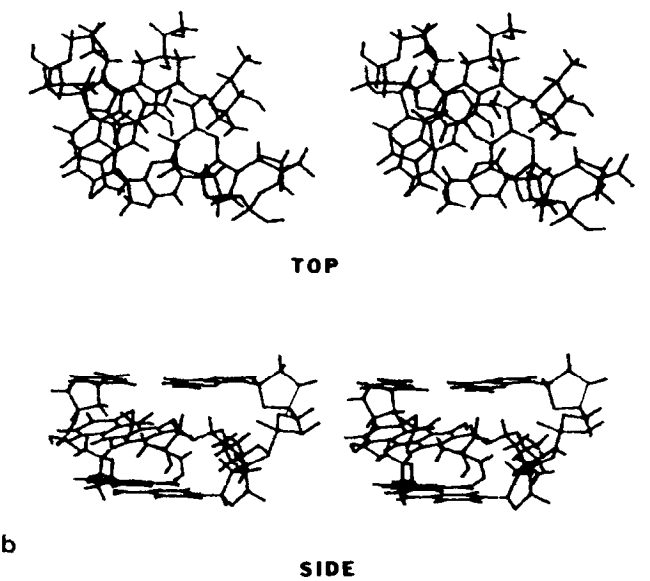


Fig. 2 Preferred mode of the intercalation of doxorubicin with (CpG) having mixed sugar ring puckering. a) Major groove b) Minor groove.

also postulate a strong electrostatic bond between the NH_3^+ group of the drug and a second nearest-neighbor nucleotide residue phosphate group. Thus these models involve a trinucleotide dimer as the intercalation "substrate". Therefore, the theoretical dinucleotide dimer structures may not be relevant to the mode of DNA intercalation. Preliminary tetranucleotide dimer calculations still support minor groove intercalation, but the results are as yet incomplete.

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