Energetics and stereochemistry of DNA complexation with the antitumor AT specific intercalators tilorone and m-AMSA

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ABSTRACT

Computations by the SIBFA method on the intercalative interaction energies of tilorone and m-AMSA with B-DNA representative oligonucleotides account for the specificity of these antitumor drugs for AT sites and minor groove intercalation. In tilorone this specificity is due to the strong preference of the side chains for the minor groove, which overcomes the preference of the chromophore for a GC intercalation site. In m-AMSA the specificity is due to the combined preference of both the chromophore and the anilino side chain for AT intercalation site and minor groove, respectively. o-AMSA is shown to manifest a similar (although significantly less pronounced specificity) as m-AMSA but a higher affinity for DNA. A comparison of the energetics and stereochemistry of intercalative binding to DNA of m-AMSA (AT minor groove specific) and 9-aminoacridine-4-carboxamide (GC major groove specific), which possess the same chromophore and differ only by the nature and position of the side chains, shows the possibility of important variations in the intercalative behaviour of chromophoric drugs as a function of the substituent groups attached to them.

INTRODUCTION

Following a widespread opinion intercalators (and among them antitumor intercalators) prefer GC rather than AT sites in DNA, while groove binding ligands (and among them antitumor ones) prefer AT sequences rather than GC sequences (1). Naturally, efforts have been therefore expanded in recent years to find or design antitumor intercalators which would be AT specific and antitumor groove binding ligands which would be GC specific (1).

Among known anti-cancer drugs, with an intercalative mode of binding to DNA, three seem to manifest specificity, or at least a significant preference, for AT sites. These are tilorone, m-AMSA and neocarzinostatin (to which may be added the bisintercalating quinoxaline antibiotic Tandem (2-4)). The determination of the factors responsible for this relatively rare specificity is of particular interest. We have carried out in a recent publication a theoretical analysis, from that point of view, of the case of neocarzinostatin (5). The same approach is extended in the present work to tilorone (I) and m-AMSA (II).

I. Tilorone

II. *m*-AMSA

III. *o*-AMSA

PROCEDURE

The procedure utilized in the present work is analogous to that used in our study on neocarzinostatin (5) and other intercalators (6-9) and will therefore not be expanded here. May we just remind that it is based on the SIBFA (Sum of Interactions Between Fragments computed Ab initio) method (10, 11) and evaluates the energy and stereochemistry of the intercalative binding of the ligands to two representative double-stranded tetranucleotides, namely: $d(\text{GCGC})_2$ and $d(\text{ATAT})_2$, the intercalation of the chromophoric part of the ligands being considered to take place between the two central base pairs.

Within this methodology, the intercalator is built of elementary constitutive fragments separated by single bonds and the variation of the intramolecular energy upon a conformational change is obtained as the variable part of the sum of interactions between the fragments expressed as :

$$(1) \quad E_{\text{conf}} = E_{\text{MTP}} + E_{\text{pol}} + E_{\text{rep}} + E_{\text{disp}} + E_{\text{tor}}$$

The intermolecular oligonucleotide-intercalator interaction energies are computed as a sum of five terms :

$$(2) \quad E_{\text{inter}} = E_{\text{MTP}} + E_{\text{pol}} + E_{\text{rep}} + E_{\text{disp}} + E_{\text{CT}}$$

In expressions (1) and (2), E_{MTP} and E_{pol} denote respectively the electrostatic and polarization contributions, computed using a multipolar expansion of the *ab initio* SCF molecular wave functions of the fragments. E_{rep} and E_{disp} are the repulsion and dispersion contributions, respectively. E_{tor} is a torsional energy contribution, calibrated for elementary rotations along C-C and C-O bonds and E_{CT} is a charge-transfer contribution (see (10,11) for details).

The DNA tetramer is built of elementary fragments, following a procedure previously utilized to compute the molecular electrostatic potential of large biomolecules (12). The fragments are the nucleic acid bases, the phosphodiester linkage and deoxyribose, with the same internal geometry as in standard B-DNA. To construct the geometry of the oligonucleotide susceptible to accept an intercalating drug, we have resorted to the conformational analysis of intercalated DNA by the AGNAS (13-15) and JUMNA (16,17) procedures. Two different unwinding angles have been reported in the literature for the intercalation of tilorone : 7° (18) and 13° (19). The latter of these angles has been used in generating the intercalation site for this drug in this investigation. For *m*-AMSA the corresponding value is 21° , following (20). The computations have been performed allowing for the presence of monovalent counter-ions, bound to the phosphates of the tetranucleotide in a configuration bridging the two anionic oxygens (following 21). Because tilorone exists in the complex in a dicationic form, no counter-ions were attached to the phosphate groups of the intercalation site (phosphates P2 and P2' of fig. 1), so as

Figure 1 : Numbering of the constituents of the investigated tetramers exemplified on d(ATAT)₂.

to maintain the ionic balance and the electrical neutrality of the complex. For the same reason, in the complex of the monoprotonated *m*-AMSA, the positive charge of the two counter-ions at the phosphates P2 and P2' was reduced to 0.5.

The search for the optimal configurations for the oligonucleotide – intercalator complexes are performed by energy-minimization (22) of the sum of E_{inter} plus E_{conf} to which is added the energy necessary to produce the unstacked intercalation site, E_{unstack} . The variables involved in the minimization are the conformational angles of the intercalator together with the intermolecular variables defining the relative orientation of the intercalator with respect to the tetramer.

RESULTS AND DISCUSSION

1) Tilorone

Tilorone binds to DNA (23) and polynucleotide systems (24, 25) in an intercalative way. Studies with synthetic polydeoxyribonucleotides indicate a preference for binding to the alternating copolymers poly(dA-dT), poly(dA-dT) and poly(dG-dC), poly(dG-dC) rather than to the complementary homopolymers poly(dG), poly(dC) and poly(dA), poly(dT) (18, 24, 25). In fact, tilorone does not seem to intercalate into the last polymer but to be only weakly bound to it, probably by outside interaction. Among the alternating copolymers the greatest extent of binding occurs with poly(dA-dT), poly(dA-dT). In so far as the copolymers may be considered as representing the B-DNA structure this result signifies the preference of tilorone for AT sequences in this conformer of this biopolymer.

The results of the computations on the energetics of binding of tilorone to the tetrameric nucleotide sequences are indicated in Table I which lists the values of the intermolecular oligonucleotide–drug interaction energies, E_{inter} , and their different components, the variation of the conformational energy of the drug in the complex with respect to the energy of its most stable conformation in the absence of interaction, E_{conf} , the values of the unstacking energies of the central base pairs with respect to their energies in the B-DNA conformation, E_{unstack} , the resulting energy balance $E = E_{\text{inter}} - E_{\text{conf}} - E_{\text{unstack}}$, and the value, δ , of the energy differences with respect to the most favorable value of E taken as energy zero.

A representation of the minor groove complex of tilorone with $d(ATAT)_2$ is given in figure 2.

The results of Table I confirm the preference of the drug for alternating AT sequences, due essentially to the electrostatic component of the interaction energy and indicate moreover a preference for minor groove intercalation. The analysis of the stereochemistry of the interaction does not provide an immediate indication on the origin of these preferences. Significantly this origin does not reside in hydrogen

Table I
Interaction energies of thiorone with tetranucleotides (see text for definitions).
Energies in kcal/mole.

	Minor groove		Major groove	
	d(GCGC) ₂	d(ATAT) ₂	d(GCGC) ₂	d(ATAT) ₂
E _{inter}	- 269.4	- 274.7	- 273.2	- 266.8
E _{MTP}	- 218.7	- 225.0	- 223.0	- 218.2
E _{pol}	- 26.2	- 25.7	- 27.0	- 25.4
E _{CT}	- 4.7	- 4.6	- 7.8	- 7.8
E _{disp}	- 64.2	- 62.2	- 97.9	- 92.6
E _{rep}	44.3	42.8	82.4	77.2
E _{conf}	16.9	17.0	35.6	37.3
E _{unstack}	22.2	23.8	22.2	23.8
6E	- 230.3	- 233.9	- 215.4	- 205.7
δ	3.6	0.0	18.6	28.2

Table II
Upper values indicate interatomic distances (in Å) between H atoms of side chains of thiorone and binding sites on the tetranucleotides. Lower values denote the angle (in degrees) centered at the H atom.

Minor groove		Major groove	
d(GCGC) ₂	d(ATAT) ₂	d(GCGC) ₂	d(ATAT) ₂
H(N ₂₄) - O ₃ .(S2)	H(N ₂₄) - O ₃ .(S2)	H(N ₂₄) - O ₁ (P1)	H(N ₂₄) - O ₁ (P1)
2.08	2.09	2.54	3.10
164.6	164.8	154.3	135.0
- O ₅ .(S3)	- O ₅ .(S3)		
2.30	2.31		
123.7	124.1		
H ₂ (C ₂₃) - O ₅ .(S3)	H ₂ (C ₂₃) - O ₅ .(S3)	H ₁ (C ₂₃) - O ₁ (P2)	H ₁ (C ₂₃) - O ₁ (P2)
2.50	2.52	2.06	1.97
116.7	116.5	164.0	175.0
- O ₁ .(S3)	- O ₁ .(S3)	H ₂ (C ₂₃) - O ₅ .(S2)	H ₂ (C ₂₃) - O ₅ .(S2)
2.40	2.40	2.71	2.77
164.3	164.2	135.1	143.2

Figure 2 : Complex of tilorone with the minor groove of $d(ATAT)_2$.

bonding to specific sites on the nucleic acid bases. Details on these bonds are presented in Table II. It is observed that the hydrogen bonds formed in the most stable arrangement (intercalation in the minor groove of the AT sequence) occur between the protonated nitrogens of the side chains and the ester oxygen of phosphate P2 and between the hydrogens of the CH_2 group next to the protonated nitrogen on the chromophore side and an ester oxygen of phosphate P3 and the ring oxygen of sugar S3. A practically identical hydrogen bonding scheme prevails for the minor groove intercalation between GC base pairs. In these circumstances one has to look for the answer to the problem by evaluating the contribution to the overall interaction energy of the two main components of the drug : the chromophore and the side chains. A detailed analysis from that point of view shows that the interaction of the chromophore with the base pairs of the intercalation site favors somewhat the GC over the AT sequence (by about 2.3 kcal/mole). This difference remains the same if all the bases of the tetramer are taken into account. On the contrary, the interaction of the side chains with the minor groove of the two sequences operates strongly in favor of the AT sequence (by about 7.3 kcal/mole). The result of these two opposite tendencies is an overall predominant affinity for the AT sequence (by about 5 kcal/mole, corresponding to the difference in the E_{MTP} term of Table I).

Globally, it is thus the preference of the side chains of tilorone for the minor groove of AT sequences that seems to be responsible for the overall preference of this drug for intercalation into these sequences rather than into GC sequences. This case illustrates thus the importance of the location of a deep

Table III
Interaction energies of m-AMSA with tetranucleotides (see text for definitions).
Energies in kcal/mole.

	Minor groove		Major groove	
	d(GCGC) ₂	d(ATAT) ₂	d(GCGC) ₂	d(ATAT) ₂
E_{inter}	- 127.2	- 133.0	- 121.6	- 119.0
E_{MTP}	- 91.0	- 94.8	- 89.4	- 85.2
E_{pol}	- 14.3	- 14.1	- 13.0	- 12.2
E_{CT}	- 3.5	- 3.0	- 3.1	- 2.6
E_{disp}	- 65.5	- 63.1	- 60.3	- 55.0
E_{rep}	47.1	42.0	44.2	36.0
E_{conf}	2.0	2.7	3.0	0.4
$E_{unstack}$	5.4	7.2	5.4	7.2
δE	- 119.8	- 123.1	- 113.2	- 111.4
δ	3.3	0.0	9.9	11.7

electrostatic potential in the minor groove of AT sequences even for intercalative association with DNA structures.

It may be interesting to add that in the intercalation site referred to above, the chromophore is oriented so that its carbonyl group points to the exterior of the complex. It was verified that the reversed orientation is significantly less stable.

2) m-AMSA

m-AMSA (amsacrine), II, is among the most conspicuous clinical drugs in the series of antitumor acridines (for a general review see 26). Its binding mode to DNA involves intercalation (20), with a marked preference for alternating purine-pyrimidine polymers and among those a moderate preference for poly(dA-dT).poly(dA-dT) over poly(dG-dC).poly(dG-dC) (27). The situation is thus altogether similar to that observed for tilorone.

The crystal structure of m-AMSA has been established both for the free base (28) and its hydrochloride salt (29). An overall feature of the structure is the nearly orthogonal orientation of the anilino ring with respect to the mean plane of the acridine ring. In the currently favored model (26) the chromophore is intercalated with the anilino ring in the minor groove, its 1' substituent pointing tangentially away from the helix.

Table IV

Upper values indicate interatomic distances (in Å) between the NH hydrogens of *m*-AMSA and binding sites on the tetranucleotides. Lower values denote the angle (in degree) centered at the H atom.

Minor groove		Major groove	
$d(GCGC)_2$	$d(ATAT)_2$	$d(GCGC)_2$	$d(ATAT)_2$
$H(N_9) - O_1(S3)$	$H(N_9) - O_1(S3)$	$H(N_9) - N_4(C2')$	$H(N_9) - O_4(T2')$
2.06	1.98	3.00	3.28
147.5	155.6	116.9	106.7
$- O_5(S3)$	$- O_5(S3)$		
3.39	3.34		
156.8	150.0		
$H(N_{10}) - N_4(C2')$	$H(N_{10}) - O_4(T2')$	$H(N_{10}) - N_3(G3)$	$H(N_{10}) - N_3(A3)$
3.90	3.84	3.16	3.13
131.0	127.7	84.1	94.0
$- O_6(G3)$	$- N_6(A3)$		
3.79	3.71		
97.4	94.9		
$- O_6(G3')$	$- N_6(A3')$		
3.90	3.86		
103.2	101.1		
		$O(-OCH_3)-H(N_4-C2')$	$O(-OCH_3)-H_1(N_6-A3)$
		2.83	2.74
		123.3	95.9
			$-H_2(N_6-A3)$
			2.83
			91.4

The results of the computations of the energetics of the oligonucleotide-drug interaction are given in Table III. They indicate the preference both for AT sequences and for the minor groove location of the anilino ring. As in the case of tilorone the analysis of the stereochemistry of the intercalated complex does not provide a straightforward indication for the reason of these preferences. The observed arrangement is stabilized by a hydrogen bond between the NH group linking the two aromatic rings and the ring oxygen of the sugar S3 (Table IV), which does not produce any marked specificity. A number of other, elongated contacts is observed (Table IV) which do not modify this situation.

A representation of the minor groove complex of *m*-AMSA with $d(ATAT)_2$ is given in figure 3.

On the other hand, a detailed analysis of the contribution to the overall interaction energy of the two main components of the drug, the acridine

Figure 3 : Complex of m-AMSA with the minor groove of d(ATAT)₂.

chromophore and the anilino side chain, similar to that performed for tilorone, shows that the intercalation of the drug in the minor groove of AT rather than GC sequences is favored in this case both by the preference of the chromophore for stacking into the former (by about 3 kcal/mole) and the preference of the anilino side chain for location in the minor groove (by about 1.6 kcal/mole). Thus the

Table V
Interaction energies (in Å⁰) of o-AMSA with tetranucleotides (see text for definitions). Energies in kcal/mole.

	Minor groove		Major groove	
	d(GCGC) ₂	d(ATAT) ₂	d(GCGC) ₂	d(ATAT) ₂
E_{inter}	- 132.9	- 137.4	- 131.4	- 128.1
E_{MTP}	- 93.6	- 97.9	- 98.9	- 93.7
E_{pol}	- 15.4	- 15.3	- 14.9	- 14.1
E_{CT}	- 2.9	- 2.5	- 3.7	- 3.5
E_{disp}	- 67.6	- 62.5	- 62.0	- 59.2
E_{rep}	46.5	40.9	48.0	42.3
E_{conf}	0.5	2.7	1.0	1.4
$E_{unstack}$	5.4	7.2	5.4	7.2
δE	- 126.9	- 127.5	- 125.0	- 119.5
δ	0.5	0.0	2.5	8.0

Table VI

Upper values indicate interatomic distances (in Å) between the NH hydrogens of o-AMSA and binding sites on the tetranucleotides. Lower values denote the angle (in degree) centered at the H atom.

Minor groove		Major groove	
d(GCGC) ₂	d(ATAT) ₂	d(GCGC) ₂	d(ATAT) ₂
H(N ₉) - O ₁ .(S3)	H(N ₉) - O ₁ .(S3)	H(N ₉) - N ₄ (C2')	H(N ₉) - O ₄ (T2')
2.38	1.89	3.50	3.46
127.2	155.4	94.7	94.2
- O ₅ .(S3)	- O ₅ .(S3)	- O ₆ (G3')	- N ₆ (A3')
3.47	3.26	3.72	3.58
166.9	148.5	77.0	84.0
H(N ₁₀) - O ₆ (G3)	H(N ₁₀) - N ₆ (A3)	H(N ₁₀) - O ₁ .(S3)	H(N ₁₀) - O ₁ .(S3)
3.61	3.91	2.94	3.07
94.4	96.7	120.5	124.5
	- O ₄ (T2')	- N ₃ (G3)	- N ₃ (A3)
	3.95	3.30	3.23
	132.7	73.7	78.5
			O(-OCH ₃)-H ₁ (N ₆ -A3)
			3.80
			111.7

factors favoring the AT specificity of m-AMSA are different from those imposing the same preference to tilorone.

3) The problem of o-AMSA

An interesting problem connected with the DNA binding and the antitumor

Figure 4 : Complex of o-AMSA with the minor groove of d(ATAT)₂.

activity of m-AMSA relates to the behaviour of its isomer o-AMSA (III) which while binding to DNA even better than m-AMSA is biologically inactive (30). This represents a particularly striking case of a situation, found also in other classes of antitumor drugs, illustrating the possible lack of correlation, or at least of a strict correlation, between DNA binding and antitumor activity.

Computations carried out for the interaction of o-AMSA with the same oligonucleotide sequences as for m-AMSA (Tables V and VI) indicate an overall similar mode of interaction and a similar (although significantly reduced) AT specificity and confirm also the higher affinity to DNA of the former (by 4.4 kcal/mole). This last feature is attributable to its slightly better intercalative penetration into the double helix and the shortening (from 1.98 Å in m-AMSA to 1.89 Å) of the same and unique efficacious H-bond indicated above for m-AMSA.

A representation of the minor groove complex of o-AMSA with d(ATAT)₂ is given in figure 4.

The inactivity of o-AMSA as opposed to the antitumor activity of m-AMSA can nevertheless hardly be interpreted in terms of a striking difference in their strength or mode of binding to DNA. A rather plausible hypothesis (27) is that the biological activity of this type of drugs is exerted through the formation of ternary complexes in which the acridine moiety serves as the DNA binding anchor, whereas the methanesulfonilide ring interacts with a second macromolecular species, possibly a protein (DNA topoisomerase). This interaction could be, for steric reasons, prevented in o-AMSA.

4) Comparison of m-AMSA with 9-aminoacridine-4-carboxamide

IV. 9-aminoacridine-4-carboxamide (9AA4C)

Another interesting comparison is that of m-AMSA with 9-aminoacridine-4-carboxamide (IV) (symbol 9AA4C), parent compound of a new class of antitumor agents with promising activity (31). The interest springs from the fact that although both compounds possess the same chromophore and differ only by the nature and position of their side chains, their intercalative properties are very different: m-AMSA, as we have just seen, shows a specificity for the minor groove of AT sequences while 9AA4C intercalates preferentially in the major groove of GC

Table VII
Interaction energies of the chromophore and of the side chains of m-AMSA and 9AA4C with the base system of the tetramers.

Compound	9AA4C			m-AMSA		
	Major groove			Minor groove		
	d(GCGC) ₂	d(ATAT) ₂	δ^*	d(GCGC) ₂	d(ATAT) ₂	δ^*
Chromophore	- 25.5	- 23.3	2.2	- 26.9	- 30.0	- 3.1
Side chains	- 22.9	- 8.9	14.0	- 2.8	- 4.5	- 1.7
Total			16.2			- 4.8

$$^* \delta = d(ATAT)_2 - d(GCGC)_2.$$

sequences (32). Moreover, the chromophore itself although similarly oriented in the two most favorable complexes, namely with the 9-amino group facing the minor groove, prefers the AT sequence in the former but the GC sequence in the latter (Table VII).

This situation may be accounted for by the differences in the detailed mode of positioning of the chromophore at the intercalation site in the two drugs, due to the effect of the interactions involving the side-chains : the chromophore is pulled towards the minor groove side of the helix in m-AMSA and to its major groove side in 9AA4C. This example illustrates thus the important phenomenon of variability of the intercalative behaviour of chromophoric drugs as a function of the groups or side chains attached to them.

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