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Groove selectivity in the interaction of 9-aminoacridine-4-carboxamide antitumor agents with DNA

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Theoretical quantitative evaluation of the intercalative binding to DNA of the new antitumor drug 9-aminoacridine-4-carboxamide indicates that, in contradiction with a recently proposed model, the compound should show specificity for interaction with the major (and not minor) groove of GC sequences.

Antitumor drug; Intercalation; Groove binding

1. INTRODUCTION

9-Aminoacridine-4-carboxamide (**I**) is the parent compound of a new class of antitumor agents with promising activity [1]. As a number of other antitumor agents it acts by intercalating into DNA, in which it binds selectively to its GC sequences [1]. In a recent attempt to establish a more precise definition of the selectivity of binding, Wakelin et al. [1] have investigated the kinetics of dissociation of calf thymus DNA complexes of **I** using the surfactant-sequestration method. The results led them to propose that the biologically significant decationic form of **I** (one proton on the ring nitrogen and the other on the terminal nitrogen of the side chain) should intercalate from the minor groove of the GC base pair. This proposal was based on the assumption [2] that "the electrostatic potential is greater in the minor compared to the major groove in B-type DNA" and on examples of other complexes (e.g. daunomycin-DNA) in which the bulk of the ligand attached to the intercalating chromophore resides in the minor groove.

None of these two arguments seems satisfactory enough to justify the proposal of the minor groove intercalation of **I**. In the first place, the estimation that the electrostatic potential is greater in the minor compared to the major groove in B-type DNA is incomplete. While this is so in AT sequences the situation is reversed in GC sequences, in which the greatest electrostatic molecular potential resides in the major groove [3,4]. In the second place, intercalators with bulk ligands attached to them, may well have these ligands located in the major as well as in the minor groove. Thus, the 'second generation' anthracyclines, mitoxantrone and anthrapyrazole, while showing a definite specificity for intercalation between GC base pairs, have their side chains located in the major groove [5,6].

For these reasons and in view of the potential importance of this new type of drug as an antitumor agent, we have carried out an explicit computation on the stereochemistry of the intercalative

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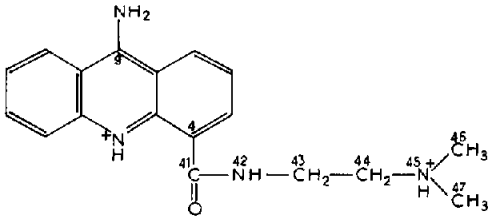

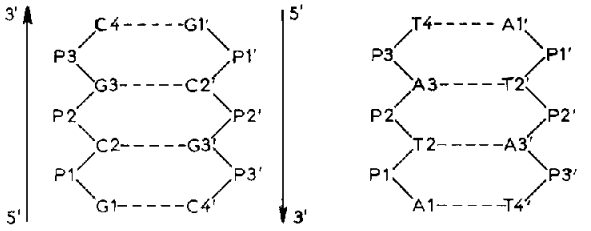

Fig.1. Base numbering in the model tetramers. Intercalation occurs between bases 2-3′ and 3-2′.

interaction of the dicationic form of **I** with tetrameric alternating AT and GC sequences in the B-DNA conformation. The method and procedure are entirely analogous to those used in our studies on the interaction with oligomeric sequences of daunomycin [7], adriamycin [8], mitoxantrone [5] and anthrapyrazole [6] and will thus not be repeated here. Fig.1 recalls the base numbering in the investigated tetramers, the intercalation occurring between bases 2-3′ and 3-2′.

2. RESULTS AND DISCUSSION

The interaction energies for the intercalation of **I** into the oligomers $d(GCGC)_2$ and $d(ATAT)_2$, in the major and minor grooves, are listed in table 1. In this table ΔE represents the intermolecular interaction energy, the components of which are also listed: electrostatic, E_{MTP}; polarisation, E_{pol}; charge transfer, E_{ct}; dispersion, D_{disp}; repulsion, E_{rep}. ΔE_{conf} represents the conformational energy modification of the ligand with respect to its intrinsically most stable form in the free state. $\delta E_{unstack}$ is the energy necessary to generate the intercalation site (in conformity with experimental indications [1] an unwinding angle of 16° was adopted for this case). $\delta E = \Delta E + \delta E_{conf} + \delta E_{unstack}$ represents the overall energy balance for the interaction and δ the relative energies of the different associations with respect to the most stable one taken as zero energy.

The results clearly confirm the GC specificity of the drug but indicate at the same time, in contrast

Table 1

Values of the intercalative interaction energies (in kcal/mol) of compound **I** in the major and minor groove of sequences $d(GCGC)_2$ and $d(ATAT)_2$ (see text for definitions)

	Major groove		Minor groove	
	$d(GCGC)_2$	$d(ATAT)_2$	$d(GCGC)_2$	$d(ATAT)_2$
ΔE	−544.2	−526.7	−504.8	−512.1
E_{MTP}	−497.9	−480.7	−451.0	−458.3
E_{pol}	−38.0	−37.5	−33.9	−32.3
E_{CT}	−11.2	−7.1	−5.4	−3.7
E_{disp}	−77.4	−83.4	−78.1	−67.6
E_{rep}	80.4	82.1	63.5	49.9
$\delta E_{conf.}$	12.8	8.6	8.1	6.7
$\delta E_{unstack}$	4.1	5.2	4.1	5.2
δE	−527.3	512.9	−492.6	−500.2
δ	0.0	14.4	34.7	27.1

to the proposal of [1], a clear-cut preference for the location of the side chain in the major groove of these sequences. It can be seen that this preference is determined essentially by the electrostatic term of the interaction energy.

A detailed analysis of the stereochemistry of the association enables one to pinpoint the essential structural features involved in the preferential binding. Table 2 indicates the main hydrogen-bonding contacts established between the drug and the oligomer for the different modes of assocations. It is easily seen that the most significant interactions occur for binding in the major groove of the GC sequence. In the optimized conformation the long axis of the chromophore is at an angle of about 30° to the long axis of the base pairs of the intercalation site. In this way the compound can strongly bind to the two strands of the tetranucleotide, the 4-carboxamide side chain binding to one and the 9-NH_2 group to the other.

The stabilization of the complexes in the major groove of $d(GCGC)_2$ and $d(ATAT)_2$ is ensured by interactions involving:

(i) A hydrogen bond between the H atom of the ammonium nitrogen N_{45} and an oxygen of the phosphate of the primed strand at the intercalation site, $O_1(P2')$ ($d_{H\text{-}O}$ = 1.58–1.77 Å);

Table 2

Interatomic distances (in Å) in the complex of compound **1** with $d(GCGC)_2$ and $d(ATAT)_2$

Major groove		Minor groove	
$d(GCGC)_2$	$d(ATAT)_2$	$d(GCGC)_2$	$d(ATAT)_2$
H(N45)–O_1(P2′) 1.58	H(N45)–O_1(P2′) 1.77 –O_3′(S-T2′) 2.95	H(N45)–O_3′(S-G3) 2.54 –O_1′(S-G3) 2.76	H(N45)–O_1′(S-T4) 2.13
H_2(C44)–N_7(G3′) 2.27	H_2(C44)–O_1(P2′) 2.36	H_1(C44)–O_1′(S-G3) 2.02	H_1(C44)–O_1′(S-A3) 2.34 –N_3(A3) 2.42 H_2(C44)–O_1′(S-A3) 2.20
H_3(C46)–N_7(G3′) 2.36	H_2(C46)–O_1(P2′) 2.81 H_3(C46)–N_7(A3′) 2.53	H_1(C47)–N_3(G3) 2.25 –O_1′(S-G3) 2.96 H_2(C47)–N_3(G3) 2.79	H_1(C47–N_3(A3) 2.88
H(N42)–O_6(G3′) 2.56 O_{41}–H_1(N_4-C2′) –H_2(N4-C2′) 2.83	H(N42)–N_7(A3′) 2.27 2.79	H(N42)–N_2(G3′) 2.87 O_{41}–H_2(N_2-G3) 2.62	
H_1(N9)–O_1′(S-G3) 2.78 H_2(N9)–O_5′(S-G3) 1.99 –O_1(P2) 2.66 –O_1′(S-G3) 2.72	H_1(N9)–O_1′(S-A3) 2.60 H_2(N9)–O_5′(S-A3) 2.01 –O_1′(S-A3) 2.41		

(ii) Hydrogen bonds between H atoms of C_{44}, C_{46} and C_{47}, on which part of the positive charge of the trimethylammonium moiety is delocalized, and N_7 of guanine (or adenine) and the oxygen of the phosphate of the intercalation site ($d_{H\text{-}N}$ or $d_{H\text{-}O}$ = 2.27–2.81 Å);

(iii) A hydrogen bond between H of N_{42} and O_6 of guanine or N_7 of adenine ($d_{H\text{-}N}$ or $d_{H\text{-}O}$ = 2.27–2.56 Å);

(iv) A hydrogen bond between the H atom of the 9-NH_2 group of I and the oxygen atoms O_1, and O_5, of the sugar linked to base G3 or A3, and O_1 of the phosphate of the unprimed strand, in the intercalation site ($d_{H\text{-}O}$ = 1.99–2.72 Å).

From table 2 it can be seen that these hydrogen bonds are stronger with $d(GCGC)_2$ than with $d(ATAT)_2$. Furthermore, there is an additional interaction in the complex with $d(GCGC)_2$ between the oxygen of the carboxamide, O_{41} and the H of the NH_2 group of cytosine C2′ ($d_{H\text{-}O}$ = 2.79–2.85 Å), which does not exist in the complex with $d(ATAT)_2$. As a result, the interaction energy is significantly more favourable for the complex with $d(GCGC)_2$ than with $d(ATAT)_2$.

As to the hydrogen-bonding interactions in the hypothetical complexes in the minor groove of the oligonucleotides they are obviously less numerous and less efficient than those occurring for interaction in the major groove.

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