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Theoretical studies of the selective binding to DNA of two non-intercalating ligands: netropsin and SN 18071

Krystyna Zakrzewska, Richard Lavery and Bernard Pullman

Institut de Biologie Physico-Chimique, Laboratoire de Biochimie Théorique, associé au CNRS, 13, rue Pierre et Marie Curie, 75005 Paris, France

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ABSTRACT

A theoretical study of the binding to DNA of netropsin and a bisquaternary ammonium heterocycle, SN 18071, is undertaken with an energy minimizing program based on empirical potential functions. The positionning of the ligand is achieved by force and torque calculations and its internal flexibility is taken into account. The binding preference of both drugs studied for the AT minor groove of B-DNA is shown to depend on both the electrostatic potential generated by the base sequence and the quality of the steric fit of the ligand in the groove. Ligand-DNA hydrogen bonds are shown to aid binding, but not to be essential in establishing binding preferences.

INTRODUCTION

Netropsin, (fig. 1), an antibiotic with antitumoral and antiviral activity, belongs to a widely studied class of non-intercalating DNA binding drugs (1, 2). It is a long, flexible molecule with positively charged ends and a number of proton donor and acceptor groups for possible hydrogen bonding spaced in-between. Numerous experimental studies (3 and references cited therein, 4, 5) have demonstrated the high specificity of this molecule for the minor groove of AT-rich DNA sequences. It has also been shown to complex with poly (dA).poly(dT), poly(dA-dT).poly(dA-dT), poly(dA).poly(dBrU), poly(dI).poly(dC) and poly(dI).poly(dBrC) (6-12) ; the presence of dG.dC pairs, on the contrary, decreases the binding (9).

Two interaction models, based on the disposition of the proton donor atoms of netropsin and the available proton acceptor atoms in the minor groove of B-DNA, have been proposed. The first, by Wartell et al. (13), postulates the bridging of the two strands of DNA across its minor groove. In this model the propioamidinium (CH_2-CH_2-$C(NH_2)_2^+$) and guanidinium (-NH-$C(NH_2)_2^+$) end groups of netropsin interact with the 5'-phosphate anionic oxygens at each end of a single dA.dT pair, the planes of the pyrrole rings are assumed to be tilted by about 30° from the plane of the base pair and a hydrogen bond is established between an amide N-H bond of netropsin and O2 of thymine.

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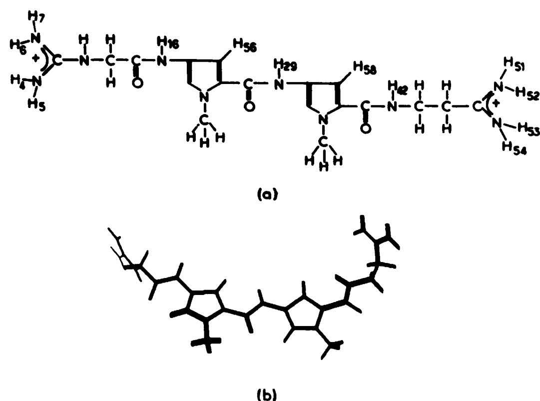


FIGURE 1. Netropsin. a) Chemical formula and atom numbering as referred to in the text. b) Minimum energy conformation in the complex with the minor groove of poly(dA).poly(dT),screened by Mg^{2+}.

The second model, which seems to be more widely accepted, was proposed on the basis of the crystal structure of netropsin by Berman et al. (14). In this model the concave side of the antibiotic carrying its hydrogen donors lies in the minor groove of DNA, approximately parallel to the sugar-phosphate backbone and spans four base pairs. The amide nitrogen atoms (numbers 16, 29, 42 in fig. 1a) can form hydrogen bonds with thymine O2's and/or adenine N3's of one strand. The propionoamidininium group is associated with the opposing strand of DNA, whereas the guanidinium residue is not involved in any specific association. A very similar model was proposed by Patel (15) based on an NMR-NOE study of the interaction of netropsin with a DNA dodecamer containing a central AATT sequence.

These models were developed on the basis of physicochemical studies and CPK model building, but the exact roles played by the charged ends of netropsin and by the hydrogen bonds remain unclear. The charged ends appear to be important since they are a common feature of many groove-binding ligands. Nevertheless, a netropsin derivative with both ends removed, although showing a decrease in binding efficiency, still complexes to poly(dA).poly(dT) (3). The ability to form hydrogen bonds has also been thought to be essential and has frequently served as a basis for theories on specific protein-DNA recognition mechanisms (16). In fact, it is not an absolute prerequisite, as it has been shown that a bisquarternary ammonium heterocycle, SN 18071 (fig. 2), which cannot form such bonds binds also to DNA and shows a similar AT minor groove specificity (17, 18).

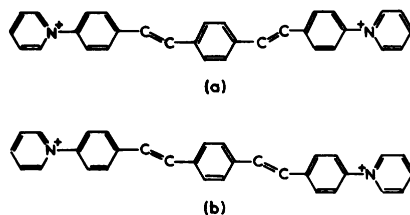


FIGURE 2. Bisquaternary ammonium heterocycle SN 18071. a) Conformation symmetric with respect to the central ring, b) Conformation with parallel C = C double bonds.

In order to help clarify the nature of this high binding specificity we have undertaken a theoretical study of the interaction by performing comparative calculations of the complexing energy of netropsin and SN 18071 with model poly(dA).poly(dT) and poly(dG).poly(dC) duplexes. Because netropsin has been shown to be specific for the B form of DNA and when interacting with A-DNA converts it to the B form (19) we have also explored this aspect of the specificity by considering the polynucleotides in both conformations. Moreover, calculations are carried out for naked DNAs in view of understanding their intrinsic preferences and also for two different models of counterion-screened DNA, so as to be closer to experimental conditions.

METHOD

An interactive program based on empirical pairwise additive energy formulae has been prepared to study the interaction of a ligand with a macromolecule through two types of molecular manipulations :

1) A study of the molecular adaptability of the ligand to a macromolecular site.

The aim of this technique is to obtain the closest fit possible for the ligand on a chosen zone of the macromolecule without introducing forbidden contacts between the two interacting species. Such contacts are considered to occur between pairs of atoms for which the Lennard-Jones energy, as defined below, exceeds a chosen positive limit. This limit can be varied at different stages of the optimization, but at the end of the procedure it is set to 5 kcal/mol.

In order to adapt the conformation and position of the ligand to the macromolecular site, we first define two target atoms within the macromolecule and two attacking atoms within the ligand. This may be, for example, two hydrogen acceptors within the macromolecule and two hydrogen donors of the li-

gand, the aim then being to minimize the distances between each such attacker-target pair. The fit is acheived by, firstly, the possibility of shifting and rotating the ligand and, secondly, by turning around any of its single bonds. In either case the motion of the ligand is guided by trigonometric calculations which attempt to optimally approach the attackers to the targets. In the case of bond rotations a cyclic procedure is employed. Initially one attacker atom is considered to be fixed in space and rotations are performed around each single bond of the ligand in order to minimize the other attacker-target distance. Each such rotation is chosen as that which, for the associated bond, best approaches the moving attacker atom to its target. If such a rotation causes close contacts, smaller rotations are tried until an acceptable conformation is found. Once all the bonds have been investigated in this manner, the role of the two attacker-target pairs is inverted and the same technique is used to optimize the position of the attacker atom which was previously considered as fixed. This entire procedure can be repeated many times and, moreover, the attacker atoms of the ligand and the target atoms of the macromolecule can be changed at will until a good fit is achieved. The conformation of the complex thus obtained is subsequently used to "seed" the energy minimizing procedure described in the following section.

2) An energy minimizing technique based on calculating forces and torques.

In order to optimize the energy of the complex we employ forces and torques acting on each atom and bond of the ligand, produced both by the macromolecule and by the ligand itself. The total force and torque acting on the center of mass of the ligand are used to calculate the direction in which the molecule should be shifted or rotated, as a rigid body, to improve the energy of the complex. Total torques acting at each atom of the ligand are also computed and guide rotations about these centers. For changes in conformations we use the torques acting on each single bond of the ligand. These torques are obtained by, firstly, calculating the forces acting on all the atoms rigidly bound to the two atoms forming the bond considered. Each of these forces produces a torque on one of the two atoms forming this bond and the summation of the components of these torques acting along the axis of the bond is used to determine which direction of rotation around the bond is most likely to improve the energy of the complex.

Since this optimization procedure is controlled interactively, any of the above data may be used at each step to attempt to improve the ligand's orientation and conformation. The program automatically rejects any change suggested which leads to macromolecule-ligand contacts or which does not lower the

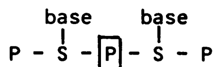
energy of the complex. Moreover, the values of the forces and torquesavailable at each step serve as a much more sensitive guide to the convergence of the conformation of the complex than does its energy alone.

The energies are calculated by empirical, atom-atom potential functions within the systematic proposed by Zhurkin et al. (20). They are expressed as a sum of Lennard-Jones functions and of a point charge electrostatic term :

$$E = \sum_{i,j} (A_{ij}/r_{ij}^{12} - B_{ij}/r_{ij}^{6} + qi\ qj\ /r_{ij})$$

where the parameters A_{ij} and B_{ij} depend on the class of the atoms i, j considered, r_{ij} is the distance between them and qi, qj are Hückel-Del Re type charges (21, 22). These have been obtained by the Hückel-Del Re procedure reparametrized to reproduce the electrostatic potentials of the nucleic acid subunits calculated with the use of Overlap Multipole Expansions (23) derived from _ab initio_ wave functions. A fit to roughly 1.5 kcal/mol at 3 Å from each of the subunits was achieved. Moreover, charge redistribution between the subunits was accounted for by calculating the reparametrized Hückel-Del Re monopoles for macromolecular fragments containing centrally the subunits whose charges are desired. Thus the charges of the bases and sugars were obtained from the fragment :

and the charges of the phosphate from the fragment :



In the second fragment it was found that the charges of the phosphate did not depend on the bases present and that the charges of the sugar differed only at the atom C1', which had a charge 0.3010 for purine bases and 0.2997 for pyrimidine bases. An average value of 0.3003 was consequently employed. In this way a net charge of -1 was maintained for a nucleotide to a precision of 0.0004e. For full details of this procedure and the reparametrization see ref. (24).

To obtain the total energy of the ligand-DNA complex we add to the ligand-DNA interaction energy the conformational energy of the ligand (with respect to its most stable conformer). On the other hand, in these calculations the geometry of the DNA molecule was kept constant. This seems to be justified

since experimental data suggest that interaction of netropsin with B-DNA causes only minor changes in DNA conformation (25).

To save computing time during the calculation of the conformational energy or forces of the ligand, only atom pairs separated by at least three bonds were considered. This corresponds to the part of its energy which depends on the conformational changes we may introduce.

The polynucleotide models used in our calculations were built from 22 5'-nucleotides producing 11 base pair oligomers. The geometry of these helices is that of Arnott et al. (26).

Two simple model screenings of the polynucleotides by conterions were explored, as in our previous calculations on electrostatic potentials (27, 28). In the first model a sodium cation was placed at the bridge position of every phosphate with a distance of 2.15 Å from the anionic oxygens (29). In the second a magnesium cation was placed at the bridge position of every second phosphate and out of step in the two phosphodiester chains, with a distance of 1.99 Å (29) from the anionic oxygens. In this way 12 magnesium cations were positioned, resulting in a total charge on the nucleic acid of +2. To compute the effect of the screening of DNA by Na^{+} and Mg^{2+} we evaluated the Lennard-Jones parameters for these cations so as to fit the rest of Zhurkin's data set and checked the energy and distances for their binding to phosphates and nucleic acid bases by comparison with ab initio calculations (29-31).

The geometry of netropsin and of SN 18071 were constructed with standard bond lengths and angles (32). The geometry of netropsin obtained in this way is similar to that found crystallographically (14) but the initial conformation adopted for the drug had both pyrrole rings coplanar and both ends of the molecule in an extended conformation. In the case of SN 18071 the three central rings were assumed coplanar, while the two terminal rings were turned at an angle of 30° in accordance with crystallographic data for several related molecules (33).

The interactive program was developed and the calculations were performed on a VAX 750 computer.

RESULTS AND DISCUSSION

Netropsin binding to B-DNA.

In fig. 3 the numbering system for the subunits of the model DNA's is shown and table 1 contains the results of calculations for the interaction of netropsin with unscreened B-DNA. The table presents the energy of the best complex for the specified groove and its two components : the interaction energy

P_{11A}
S_{11A} — A_{11} — T_{11} — S_{11T}
P_{10A} P_{11T}
S_{10A} — A_{10} — T_{10} — S_{10T}
P_{9A} P_{10T}
S_{9A} — A_{9} — T_{9} — S_{9T}
P_{8A} P_{9T}
5' S_{8A} — A_{8} — T_{8} — S_{8A} 3'
P_{7A} P_{8T}
S_{7A} — A_{7} — T_{7} — S_{7T}
P_{6A} P_{7T}
S_{6A} — A_{6} — T_{6} — S_{6T}
P_{5A} P_{6T}
S_{5A} — A_{5} — T_{5} — S_{5T}
3' P_{4A} P_{5T} 5'
S_{4A} — A_{4} — T_{4} — S_{4T}
P_{3A} P_{4T}
S_{3A} — A_{3} — T_{3} — S_{3T}
P_{2A} P_{3T}
S_{2A} — A_{2} — T_{2} — S_{2T}
P_{1A} P_{2T}
S_{1A} — A_{1} — T_{1} — S_{1T}
P_{1T}

FIGURE 3. Notation scheme for the poly(dA).poly(dT) model studied. An analogous notation is used for poly(dG).poly(dC).

and the conformational energy of the ligand (with respect to the minimum energy conformation for the free molecule). We also indicate the electrostatic and Lennard-Jones terms of the interaction energy and the last column lists the intermolecular hydrogen bonds (defined as such when proton donor-proton acceptor atom pairs are separated by less than 2.5 Å).

As it can be seen from table 1 the lowest energy complex is found in the minor groove of the AT sequence. It is the most favoured one by both the electrostatic and Lennard-Jones energy terms. Second best is the minor groove of the GC sequence, weaker by 45 kcal/mol. For both sequences the locations in the major groove are disfavoured.

For the unscreened nucleic acids the energy is dominated by the electrostatic term. One could expect therefore a certain correlation between this energy and the molecular electrostatic potentials on the surface envelopes of the corresponding polynucleotides. The latter have been calculated in our laboratory (34) and the results have shown that the deepest potentials on such envelopes are situated in the minor groove for the AT sequence, but in the major groove for the GC sequence. The expected correlation is thus observed for AT sequence, but not for the GC sequence. This can be attributed to a better fit of netropsin into the depths of the minor groove for both sequences. This good fitting is illustrated by the Lennard-Jones energy term, shown in Table 1 which, al-

TABLE 1

Netropsin binding to unscreened B-DNA (kcal/mol)

SEQUENCE	GROOVE	ENERGY OF THE COMPLEX	INTERACTION ENERGY			LIGAND CONFOR-MATIONAL ENERGY	LIGAND-DNA HYDROGEN BONDS
			TOTAL	ELECTRO-STATIC	LENNARD-JONES		
AT	Min.	-1190.6	-1198.8	-1158.5	-40.3	8.3	H5 - Thy_9 (O2) H16 - Ade_7 (N3) H42 - Ade_5 (N3) H51 - Thy_6 (O2) H51 - Sug_{6T}(O1') H52 - Thy_5 (O2)
	Maj.	-1079.4	-1096.5	-1077.9	-18.6	17.2	H52 - Ade_4 (N7) H5 - Thy_6 (O4)
GC	Min.	-1145.6	-1154.8	-1121.0	-33.8	9.3	H5 - Sug_{10}(O1')
	Maj.	-1108.9	-1126.1	-1111.9	-14.2	17.2	H52 - Gua_4 (N7)

though small by comparison with the electrostatic term, is much stronger for the minor than for the major grooves, notably in the case of the AT sequence. This Lennard-Jones energy is a good measure of the quality of steric fit between the ligand and the macromolecule because of its strong distance dependence which causes it to become favourable only for very close interactions, but also to rapidly become extremely repulsive if any close contacts are produced.

From the last column in table 1 we can see that when complexing in the minor groove of the AT sequence the molecule is deeply imbedded, forming six hydrogen bonds between its terminal and amide hydrogens and the Adn(N3)'s, Thy (O2)'s and a Sug(O1'). A much looser arrangement is found for the GC minor groove, due apparently to the steric and electrostatic influence of the amino groups of guanines. Only one hydrogen bond was found between the guanidinium end and a Sug(O1').

The interaction of netropsin with screened B-DNA has been investigated, as described in Methods, using either Na^+ or Mg^{2+} counterions. The results for the two models are very similar, with the exception of the absolute values of the energies. We shall thus present here only those obtained for Mg^{2+} (Table 2).

By comparison with Table 1 it can be seen that the order of preference for the different grooves is unchanged with respect to naked DNA : the minimum

TABLE 2

Netropsin binding to B-DNA screened by Mg^{2+} (kcal/mol)

SEQUENCE	GROOVE	ENERGY OF THE COMPLEX	INTERACTION ENERGY TOTAL	ELECTRO-STATIC	LENNARD-JONES	LIGAND CONFOR-MATIONAL ENERGY	HYDROGEN BONDS
AT	Min.	-153.8	-161.4	-118.8	- 42.6	7.6	H4 - Thy_{10} (O2) H5 - Thy_9 (O2) H9 - Thy_9 (O2) H16 - Ade_7 (N3) H51 - Thy_6 (O2) H52 - Thy_5 (O2)
	Maj.	- 37.3	- 52.3	- 32.9	- 19.4	15.0	H52 - Ade_4 (N7)
GC	Min.	-102.5	-110.8	- 75.1	- 35.7	8.3	H5 - Cyt_9 (O2) H51 - Sug_6 (O1') H52 - Cyt_5 (O2)
	Maj.	- 66.9	- 84.1	- 68.5	- 15.6	17.2	H52 - Gua_4 (N7)

energy complex occurs with the minor groove of the AT sequence, followed by the minor groove of the GC sequence, weaker by 50 kcal/mol. The values of the interaction energies are however strongly reduced as compared to those of Table 1, due to a reduction in the electrostatic energy, while the Lennard-Jones term is, on the contrary, somewhat stronger. The overall preference for the minor over the major groove is slightly increased upon screening. The optimal positions of netropsin in different grooves are very similar to those found for the unscreened nucleic acids.

In Fig. 4 we present a computer graphic of netropsin bound to the minor groove of the AT sequence of Mg^{2+} screened DNA. Fig. 4a gives the view along the helical axis, showing that netropsin is virtually invisible, being well imbedded in the groove while fig. 4b shows the side view, with the two pyrrole rings visible centrally and the end groups wrapped around the DNA groove. Apart from the hydrogen bonds mentioned in table 2 there are few other interesting close approaches. The amide hydrogens 29 and 42 do not form hydrogen bonds according to our geometrical criterium, but both of them are located in the vicinity of Adn(N3')s. H29 is distant 2.80 Å from Adn_6 and H42 is distant 2.85 Å from Adn_5. The pyrrole hydrogens 56 and 58 are close to HC2 of Adn_7 and Adn_6,

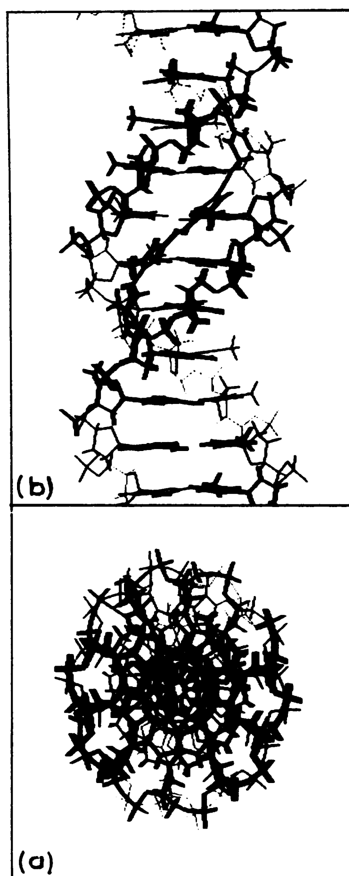


FIGURE 4. Netropsin complexing with the minor groove of poly (dA).poly(dT) in the B conformation, screened by Mg^{2+}.
a) Viewed along the helical axis,
b) viewed perpendicularly to the helical axis.

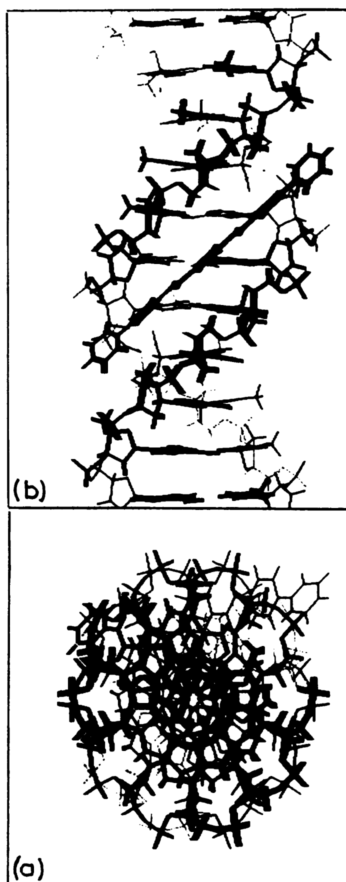


FIGURE 5. SN 18071 complexing with the minor groove of poly (dA).poly(dT) in the B conformation, screened by Mg^{2+}.
a) View along the helical axis,
b) view perpendicular to the helical axis.

by 2 Å and 2.7 Å, respectively. These two contacts were observed by Patel (15). Using NOE measurements he proposed these distances to be 2.5 Å.

A further interesting feature of our complex is also that both charged ends of netropsin are well in the groove, forming hydrogen bonds with Thy(O2)'s. We were unable to produce any complex which combined this in-groove position of the molecule with a direct interaction of its charged ends with the phosphates,

TABLE 3

SN 18071 binding to unscreened B-DNA (kcal/mol)

SEQUENCE	GROOVE	ENERGY OF THE COMPLEX		
		TOTAL	ELECTROSTATIC	LENNARD-JONES
AT	Min.	-1057.4	-1031.1	- 26.3
	Maj.	-1008.3	- 989.2	- 19.1
GC	Min.	-1012.3	- 993.2	- 19.0
	Maj.	-1031.5	-1009.5	- 22.0

in correlation with conclusions of (12). It is interesting to compare the conformation of netropsin extracted from the optimal complex, shown in fig. 1b, with the crystal structure determined by Berman et al. (14). The main feature of the crystal structure is the tilt between the two pyrrole rings determined to be 26°. In our complex the netropsin rings are skewed by 36°. The propioamidinium end is almost perpendicular to the rest of the molecule in both our conformation and in the crystal structure. On the other hand, in contrast to the crystal conformation, where the guanidinium end is approximatively coplanar with the adjacent pyrrole ring, our guanidinium end is considerably twisted. This represents the greatest difference between Berman's model, in which the guanidinium end is not involved in any specific interaction and our complex, in which three hydrogen bonds are formed involving two of the amine hydrogens and the amide hydrogen H9.

SN 18071 binding to B-DNA.

To understand better the respective roles played by the charged ends and the hydrogen bonds present in many groove binding drugs we performed a similar study of the complexation of DNA with the bisquarternary ammonium heterocycle SN 18071 (17) (fig. 2). This drug, consisting of five six-membered rings (two of which at the terminals of the molecule are positively charged) and two C = C double bonds, has no possibility of hydrogen bond formation. It can apparently adopt two major conformations (shown also in fig. 2). For an isolated molecule these two conformations have virtually the same energy but, as our preliminary studies showed a much better fit to DNA's curved grooves for the form (b), we limited our calculations to this form. Moreover, since this molecule is conjugated throughout its length it should be relatively rigid, hence table

TABLE 4

SN 18071 binding to B-DNA screened by Mg^{2+} (kcal/mol)

SEQUENCE	GROOVE	ENERGY OF THE COMPLEX		
		TOTAL	ELECTROSTATIC	LENNARD-JONES
AT	Min.	-42.1	-14.3	-27.8
	Maj.	20.4	40.6	-20.16
GC	Min.	- 7.9	13.1	-21.0
	Maj.	- 6.6	14.7	-21.3

3 which presents its complexing energies for the different grooves of the unscreened B-DNA contains only the intermolecular terms. As it can be seen in this table, the AT minor groove corresponds to the minimum energy of the complex, stronger by 27 kcal/mol than the GC major groove. There is thus more correlation with the electrostatic potentials of the surface envelope of DNA for SN 18071 binding than for netropsin, for which the minor groove was favoured over the major groove for both sequences. This is due to the fact that SN 18071, less flexible than netropsin, cannot adapt as well its conformation to the DNA grooves. The minor groove of the AT sequence is, however, quite clearly privileged by the Lennard-Jones term, demonstrating an intrinsically good steric fit with this sequence.

The results obtained for SN 18071 complexing with the screened polynucleotides are given in Table 4, again for the model with Mg^{2+} counterions.

Once again the best binding position is obtained for the minor groove of the AT sequence, stabilized by both electrostatic and Lennard-Jones terms. It is followed by the GC grooves, both roughly 34 kcal/mol weaker than the optimum complex. The least favourable binding position is the AT major groove.

In fig. 5 we present the optimal complex of SN 18071 with the minor groove of the AT sequence. The view along the helical axis shows that the molecule, being rigid, cannot completely follow the curvature of the groove and one of its ends remains visible ; the side view shows its location almost central in the minor groove.

The results obtained for SN 18071 demonstrate that the formation of hydrogen bonds is not necessary to obtain a preference for the minor groove of the AT sequence of B-DNA. It seems that if a relatively good steric fit can be ob-

tained in the minor groove the substrate will be sufficiently stabilized by the favourable potentials generated by the AT sequences. This result verifies the dominant role of these potentials in such an interaction as proposed by B. Pullman and A. Pullman (35, 36).

Netropsin and SN 18071 hypothetical binding with A-DNA.

In order to verify the selectivity of netropsin for B-DNA and its inability to form a stable complex with A-DNA, we have investigated a hypothetical association of both ligands with this latter form. The results for such an interaction of netropsin with the unscreened model polynucleotides in the A conformation show that the lowest energy complex would occur in the major groove for both the AT and GC sequences, the drug spanning the groove which is wide enough to accomodate it, its two charged ends forming bonds with the phosphate anionic oxygens on the 5'-ends of the Ade_7-Thy_7 or Gua_7-Cyt_7 base pairs. The energies so obtained are about 100 kcal/mol greater than the maximal interaction energies obtainable with B-DNA. This result correlates with the study on the distribution of the molecular electrostatic potential in A-DNA (34), which showed for the naked acid the location of the deepest potential in the major groove, independently of the base sequence studied.

The results for the A-DNA screened by sodium and magnesium counterions show the dramatic effect of screening on their complexing abilities. As in the case of the electrostatic potentials (27, 28) a clear inversion of groove preference is observed. The AT minor groove now becomes the best binding site, and in the case of Mg^{2+} screening it gives the only complex having a negative binding energy. The value of this energy is, however, extremely small (- 11.1 kcal/mol), much smaller than the similar value for the binding with the B-form of DNA (- 153.8 kcal/mol).

These data may be considered as showing the limited practical significance in this case of the results obtained when comparing different allomorphs of the naked nucleic acids, however important this may be for indicating the intrinsic preferences of these macromolecules. They also show that in conditions considerably closer to experimental circumstances (screened nucleic acids) the stability of the association with the B forms exceeds enormously that with the A form, a situation which makes it understandable that when put in the presence of A-DNA netropsin converts it into the B form.

Computations for SN 18071 have given very similar results.

CONCLUSIONS

Our calculations for the two non-intercalating groove binding drugs, netropsin and SN 18071, yield an explanation of the experimentally observed pre-

ference of these molecules for the minor groove of the AT sequence of B-DNA. The calculated energies show the considerable importance of the electrostatic term of the interaction energy, but indicate that the AT minor groove is also the most stable from the point of view of the Lennard-Jones interaction energy and thus sterically favourable. Hydrogen bonds between the ligand and the groove atoms are profitable in generating the selectivity, but not essential, as shown by the results for SN 18071 which is unable to form such bonds.

When comparing the energy difference between the best complex and the second best for the two drugs one can see that this value is bigger for netropsin than for SN 18071 in either the screened or unscreened cases. This correlates with the higher selectivity for the AT sequence of netropsin than of SN 18071, found experimentally (17, 18).

As a limitation of the present study, it should be recalled that flexibility of the DNA has not been taken into account. However, as mentioned previously, the experimental indications are that the perturbation of DNA structure due to the binding of these drugs is rather small. It is thus unlikely to disturb the clear order of complexation energy with the different DNA conformations, grooves and sequences, that we have determined. As a second limitation it should be indicated that, as generally in this type of study, which refers to gas-phase interactions, the absolute magnitudes of the energy values calculated should not be correlated directly with experimental enthalpies of complexation of these drugs in solution, which may be expected to be significantly smaller. In fact in this case, this energy has just been determined for netropsin as being - 10.7 kcal/mol (12). To obtain theoretically such quantitative values an inclusion of the effects of the solution environment of the complex would be necessary. Some approximate treatments (see eg. [37]) indicate that the reduction would probably be of the appropriate order of magnitude. However, it is unlikely that this refinement would modify the relative order of the preference obtained.

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