THE THEORETICAL STUDY OF THE INTERACTION OF SOME ANTHRACYCLINE DRUGS WITH NUCLEIC ACIDS

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abstract: The interactions of many important anticancer drugs with DNA play an important role in their biological functions. In fact, DNA can be considered as a macromolecular receptor for those drugs. There are several classes of DNA-binding anticancer drugs. In the present investigation, molecular modeling has been employed to study the interaction of three compounds of the anthracycline group with different mono- and double- stranded DNA sequences in order to evidence the sequence specificity of the drugs and explore the binding modes for this class of compounds. The modeling data are in good agreement with the physical data showing that the compounds intercalates DNA with the anthraquinone chromophore intercalated between the base pairs and the amino-sugar placed in the minor groove. The results outline the reduced affinity to adenine and thymine sequences, the increased affinity towards cytosine and guanine sequences and the differences in the relative contributions of the electrostatic and van der Waals terms to the total binding energy.

Introduction

Anthracycline antibiotics represent an important class of anticancer compounds that currently are being used for the treatment of different cancers. Among them daunomycin, doxorubycin and epirubycin are the best-known representatives of the anthracycline group. It has been shown that the biological activities of the anthracycline antibiotics are likely to be associated with their DNA binding properties. Previous experimental studies [1-6] have pointed out a complex nature of the binding process, evidencing besides intercalation an external cooperative interaction. The dependence of the binding constants on the ionic strength of the medium allowed the partition of the binding free energy in electrostatic and non-electrostatic contributions. It was found that the non-electrostatic contribution prevails.

In the present paper, the following aspects of the theoretical modeling of the interaction of the anthracycline drugs with different DNA sequences have been studied:

- The estimation of the optimal geometry of the drug – DNA complexes,
- The determination of the binding energy of the docked complexes and its partition in the van der Waals and electrostatic contributions,

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- evidencing the sequence specificity of the drugs through the usage of some model single and double stranded DNA sequences containing the AAAAAA, TTTTTT, CCCCCC and GGGGGG and mixed purine and pyrimidine sequences.

Materials and methods

The structures of the drugs and the DNAs were built within the HyperChem (version 5.02) program and optimized by the semiempirical AM1 method (parameters: SCF control of 0.01, RHF spin pairing, Polak-Ribiere optimizer, RMS gradient 0.01kcal/mol·Å for drugs and 0.1kcal/mol·Å for sequences of DNA). The calculations on the drug – DNA complexes were performed in vacuo by both the Molecular Mechanics (MM+ force field) and the AM1 methods. The optimization criteria were 0.1kcal/mol·Å for the MM method, 0.3 kcal/mol·Å for complexes of the drugs with sequences of mono-stranded DNA and 1kcal/mol·Å for complexes of the drugs with sequences of double-stranded DNA for the AM1 method.

Results and discussion

The chemical structure of anthracycline antibiotics (Figure 1) includes two structural constituents: a planar anthraquinone ring and an amino-sugar. The three drugs differ by the position of the hydroxyl group attached to C4/ of the amino-sugar and the nature of the substituent R attached to ring A. The hydroxyl group attached to C4/ of the amino-sugar is in axial position in daunomycin and doxorubycin and in equatorial position in epirubycin.

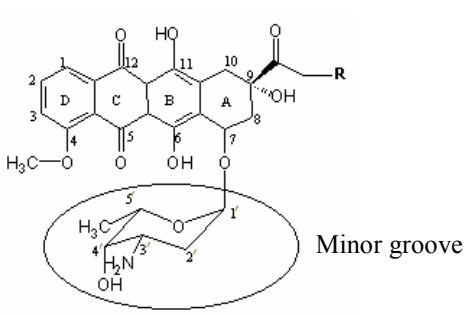

Fig. 1: *The molecular structure of anthracycline antibiotics: daunomycin (R – H), doxorubycin (R – OH) and epirubycin (R – OH)*

The equilibrium geometries of the drugs (optimized by the AM1 method) obtained in a previous study [7] were used.

The anthracycline antibiotics bind to DNA by the process of intercalation. The fundamental aspects of the physical chemistry of their binding to DNA have been well characterized [1-2]. A first detail over the interaction of an anthracycline drug with DNA was provided by the near-atomic (1.2 Å) resolution structure of the 2:1 complex between daunomycin and d(CGTACG) [8]. That structure showed that the drug molecules bind to DNA by

intercalating the chromophore between the CpG bases at both ends of a distorted B-DNA double helix. The elongated aglycon chromophore penetrates the DNA double helix with the ring D protruding into the major groove and the amino sugar lying in the minor groove. Whether a drug binds preferentially to particular DNA sequences or not has been a vexing question and the published literature is both confusing and contradictory [9-12]. An interesting sequence dependence on the binding of the amino-sugar to the AT base pair outside the intercalation site was suggested [12]. In the daunomycin/ doxorubycin with d(CGATCG) complexes there are additional direct hydrogen bonds between the positively charged N3′ amino group in the sugar and the O2 of both C11 and T10 residues of DNA. This suggests that daunomycin/ doxorubycin may bind to 5′-CGA sequence slightly better than to 5′-CGT sequence.

Other indication of the sequence specificity of the daunomycin binding to DNA was provided by the theoretical studies of the Pullman group [13]. These studies suggested that a triplet sequence was recognized as a preferred site by the daunomycin with the sequences 5′ACG and 5′TCG being the most energetically favored sites. This DNA base triplet specificity agrees with the prediction from the experimental [14] and theoretical studies [15]. In these conditions, we have chosen some model mono- and double- stranded DNA containing three base pairs. The nucleic acids were optimized by both MM and AM1 methods. Two further simplifying hypotheses were used: the solvent effect was not considered and the charge of the phosphate groups was neutralized with hydrogen atoms.

The optimized conformers of the drugs and the DNAs were further utilized in the optimizations of the drug - DNA complexes. The starting structures of these complexes were built by the docking procedure. Initially, several restraints were imposed, so that the aglycon fragment of the drug intercalate between the base pairs of DNA. We have considered that the rings B and C are stacked in the duplex, the ring D protrudes into the major groove of the double helix, whereas the ring A and amino-sugar lies on the minor groove side of the DNA helix [3,16-18]. After optimizing the complexes, the restraints were eliminated and the complexes were optimized again. The optimized geometries of two drug – DNA complexes are presented in figures 2 and 3.

Considering a thermodynamic cycle, the intercalation process can be characterized by three energies [19-20]:

- The interaction energy defined as the difference between the energy of the optimized complex and the sum of the totally optimized free drug – DNA energies.

$$E_{\text{int}eraction} = E_{complex} - (E_{drug} + E_{DNA})_{optimized} \quad (1)$$

- The binding energy defined as the difference between the energy of the optimized complex and the sum of the energies of the drug and the DNA, both frozen in the conformations found in the complex.

$$E_{binding} = E_{complex} - (E_{drug} + E_{DNA})_{frozen\ in\ complex} \quad (2)$$

This energy represents the sum of the van der Waals and the electrostatic contribution.

- The perturbation energy of both partners, due to the formation of the complex, is represented by the difference between the first two energies.

$$E_{perturbation} = E_{interaction} - E_{binding} \quad (3)$$

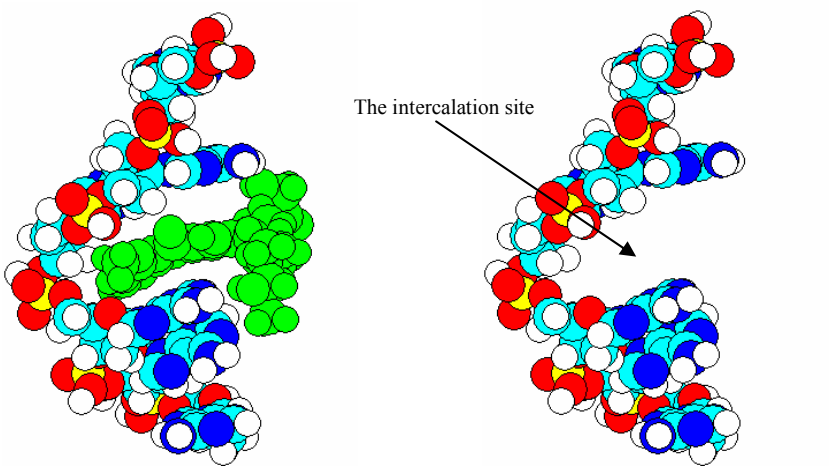

Fig. 2: *The optimized geometries of epirubycin – AAAAAA complex (a).*
The intercalation site of the drug to mono-stranded DNA (b).

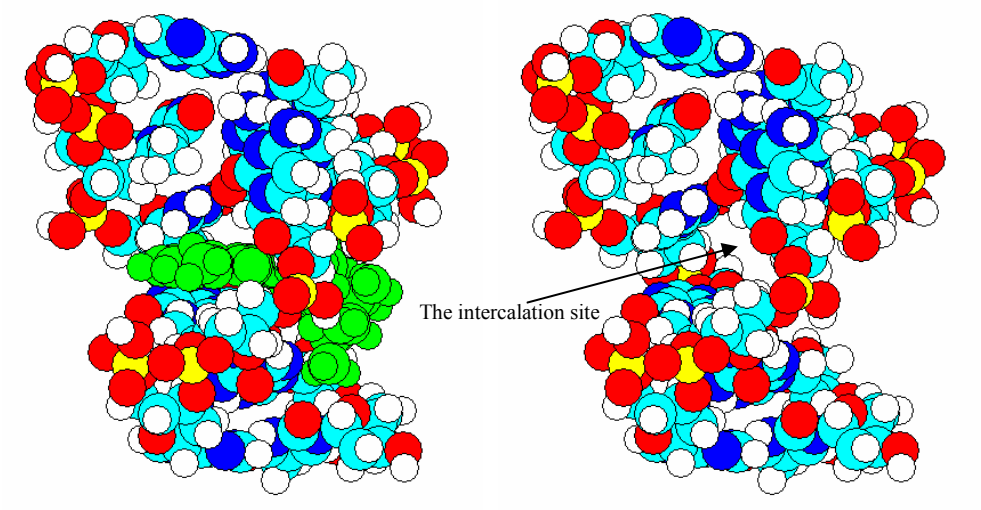

Fig. 3: *The optimized geometries of epirubycin – ATCGAT-TAGCTA complex (a).*
The intercalation site of the drug to double-stranded DNA (b).

The values of the binding energies calculated by MM and the van der Waals contributions to the binding energy are presented in Table 1. In all cases, the binding energies have negative values reflecting the drug - DNA interaction. The results underline the significant van der Waals contribution to the binding energy and, consequently, the low percentage of the electrostatic interactions, in agreement with our previous experimental data.

Table 1. MM results of drug – DNA interaction

Drug	Doxorubycin		Epirubycin	
DNA	$E_{binding}$, kcal/mol	% van der Waals	$E_{binding}$, kcal/mol	% van der Waals
AAAAAA	-38.69	71.75	-31.13	82.54
TTTTTT	-20.94	94.36	-19.71	94.82
ATATAT	-20.41	82.34	-22.79	94.08
CCCCCC	-22.17	85.27	-19.75	63.39
GGGGGG	-22.26	70.89	-20.69	82.97
CGCGCG	-17.94	82.75	-19.02	70.89
ATCGAT	-25.03	81.59	-32.42	73.83
CGATCG	-20.36	84.19	-28.46	79.95
AAAAAA-TTTTTT	-9.09	54.75	-18.36	46.72
ATATAT-TATATA	-9.36	52.06	-14.04	59.44
CCCCCC-GGGGGG	-24.95	55.46	-13.79	51.14
CGCGCG-GCGCGC	-54.27	92.52	-54.61	85.69
ATCGAT-TAGCTA	-54.58	87.14	-58.81	78.28
CGATCG-GCTAGC	-51.59	92.66	-59.88	83.29

The binding energies obtained by the AM1 method are presented in Table 2.

Table 2. Results of drug – DNA interaction obtained by the AM1 method

	$E_{binding}$, kcal/mol	
DNA sequence	Doxorubycin	Epirubycin
AAAAAA	-11.55	-5.28
TTTTTT	-7.58	-4.14
ATATAT	1.74	1.61
CCCCCC	-0.44	-8.01
GGGGGG	-3.66	-6.85
CGCGCG	-3.98	-1.39
ATCGAT	-10.11	-3.31
CGATCG	-0.21	-4.79

For the single-stranded DNA, negative values of the binding energies were obtained. In the case of ATATAT sequences, the binding energies were positive likely due to the rough approximations used.

Conclusions

Our molecular modeling points out that the anthracycline - DNA complexes are stabilized mainly by van der Waals forces involving the aromatic chromophore and the DNA bases and that the electrostatic term brings only a minimal contribution (<20%) to the binding energy. As a result of the drug – DNA interaction, only the DNA structure is significantly perturbed, the structure of the drug being practically unchanged.

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