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Analysis of actinomycin D–DNA model complexes using a quantum-chemical criterion: Mulliken overlap populations

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Abstract

The binding of the antitumoral drug actinomycin D to single- and double-stranded DNA was investigated using molecular modeling in the frame of MM^+ molecular mechanics and AM1 semi-empirical method. Two other programs, especially conceived to analyze hydrogen-bonding patterns in biological macromolecules, HBexplore, based on geometrical criteria and SHB_interactions, based on quantum-chemical criteria (Mulliken overlap populations), were also used. The results account for the non-cooperative intercalative binding process previously investigated, and outline the contribution of specific hydrogen bonding as well as C–H···O(N) and other atom–atom intermolecular interactions to the stabilization of the actinomycin D–DNA complexes. They also support the hemi-intercalation model proposed in literature for the actinomycin D–ssDNA complex.

Keywords: Actinomycin D; SHB_interactions; Nucleic acids; Hydrogen bonds; Overlap populations

1. Introduction

Actinomycin D is an antibiotic used clinically for the treatment of highly malignant tumors, such as Wilms' tumor [1] and gestational choriocarcinoma [2], and has also been used in combination with other antitumoral agents to treat high-risk tumors [3,4]. Structurally, actinomycin D contains a 2-aminophenoxazin-3-one chromophore and two identical cyclic pentapeptide lactones (Fig. 1). The pharmacological activity of actinomycin D is generally attributed to its tight interactions with DNA, which cause inhibition of transcription elongation by the blockage of RNA polymerase [5].

Actinomycin D binds to double-stranded (ds) DNA by intercalation of the planar chromophore, preferably at the GpC sequence, with the two-pentapeptide rings resting on the minor groove. A model of actinomycin D–dsDNA complex has been generally accepted, in which the phenoxazone chromophore is intercalated between the G·C and C·G base pairs, forming strong hydrogen bonds in the minor groove between the guanine 2-amino groups and the carbonyl oxygen atoms of the L-threonine residues of the cyclic pentapeptides [6,7]. Additional stabilizations are derived from hydrophobic interactions between groups on the pentapeptides and sugar residues, and from other specific weaker hydrogen bonds [8,9]. Also, it was observed that actinomycin D binds tightly and specifically to single-stranded (ss) DNA and this binding, described in terms of a hemi-intercalation model, may be involved in the termination of the transcription by the drug [10–13]. More recent studies indicate that actinomycin D can bind to consecutive GpC sites, separated by a T:T mismatched base pair inducing some conformational distortions, particularly kinks in the helix, unwinding of the base pairs and widening of the minor groove [14]. Although other antitumoral drugs with better therapeutic properties and less cardiotoxicity have been used in the last years, actinomycin D with two polypeptide chains attached to a phenoxazone chromophore, is still of interest as model compound for studying nucleic acids–protein interactions.

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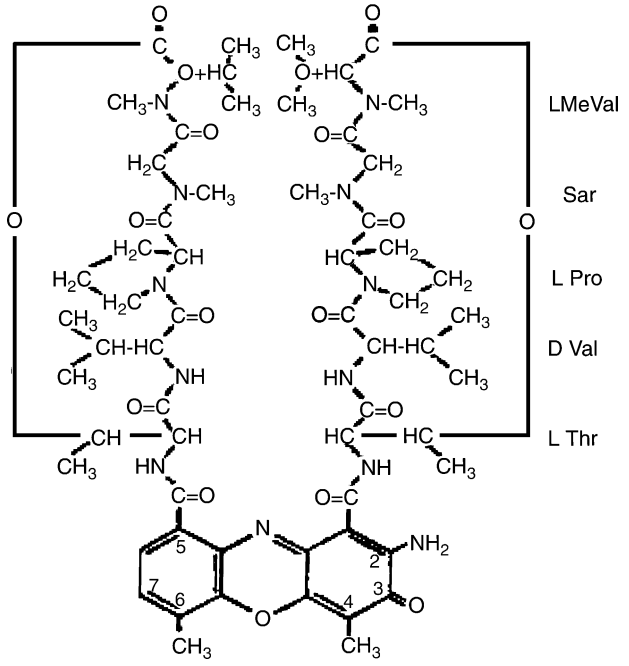

Fig. 1. The molecular formula of actinomycin D.

In our previous studies, Scatchard plots for binding of actinomycin D to dsDNA and ssDNA using absorption and fluorescence spectroscopy, indicate the presence of three distinct processes: (a) a cooperative process at low P/D (the ratio between the polymer and drug concentrations) values, assigned to the external binding of actinomycin D to nucleic acids; (b) a non-cooperative process assigned to the intercalation of the phenoxazone chromophore of the drug between the base pairs of the nucleic acids and (c) a cooperative process at high P/D values, which could not be assigned [15,16]. Also, other experimental data have pointed out a major non-electrostatic contribution to the binding free energy [17], as well as the formation of specific hydrogen bonds between the drug and the base pairs of nucleic acids [6,7,13].

The objective of the present work is to analyze the contribution of the potential hydrogen bonds and other atom–atom intermolecular interactions to the stability of the actinomycin D–DNA intercalation complexes, using the Mulliken overlap populations as quantum-chemical criterion, besides geometrical criteria. To this purpose, the following methods were employed: molecular mechanics, semi-empirical methods and a specific tool for identifying and analyzing hydrogen bonding patterns in biological macromolecules based on geometrical criteria (HBexplore) [18], as well as an original program (SHB_interactions) [19], using quantum-chemical criteria in the study of these interactions.

Quantum-chemical methods (ab initio with inclusion of electron correlation) were generally used in the study of base–base and deoxyribose–base-stacking interactions in B-DNA and Z-DNA, for which older quantum-chemical studies do not provide a correct description [20]. Other high-level quantum-chemical calculations, including H-bond interactions with different acceptor and donor atoms, were performed on small molecules or on minimal structural unities suitable to model fragments of nucleic acids, and the results are extrapolated to biopolymers [21–23].

In the present work only the intercalation complexes corresponding to process (b) in our previous experimental results were addressed to. Processes (a) and (c), which present cooperativity according to our experimental data, were not explicitly considered in the present work. Cooperativity effects were only very recently taken into account in the formation of intercalation sites [24]. Published molecular dynamics simulations on other drug–DNA complexes describe cooperative effects on the formation of intercalation sites and report that the introduction of the second intercalation site decreases the energy cost by about 5 kcal/mol. The authors claim these calculations to be up to now the most complete ones and to consider hydration effects for the first time [24]. Influence of hydration or cations was also only recently considered in molecular dynamics simulation of the nucleic acids–protein complexes [24,25]. Therefore, the assignment of cooperative processes (a) and (c) in the frame of the present methods (using comparative study of SHB_interactions program and molecular mechanics methods) as well as hydration effects, will be considered in a further step.

2. Computational details

The structure of the drug was built using the HyperChem Release 6.01 program and optimized by the semi-empirical AM1 method (EF optimization algorithm with RMS gradient of 0.1 kcal/mol Å), followed by MM^+ conformational search. The structures of the drug–nucleic acids complexes were optimized by molecular mechanics (MM^+ force field, Polak-Ribiere optimization algorithm with RMS gradient of 0.05 kcal/mol Å).

In order to identify and analyze intermolecular interactions, an original program SHB_interactions [19], was especially conceived. This program is based on Extended Hückel (EH) calculation and uses the Mulliken overlap populations as a quantitative quantum-chemical criterion. Differing from the previous HBexplore [18] program, based on geometrical criteria, that outline only the potential hydrogen bonds, this program allows an estimate of the contribution of every atom–atom intermolecular interaction to the stabilization of the drug–nucleic acid complex. The choice of the overlap population as a quantitative quantum-chemical criterion, able to measure the strength of atom–atom intermolecular interactions, is justified also qualitatively: the more positive is the electronic population of atomic overlap distribution $\chi_A^* \chi_B$ (A and B are two neighboring nuclei), the greater the overlap distribution contributes to the atom–atom interaction, chemical bond being a classical example.

EH method was chosen because this method is run time efficient and provides, due to our algorithm, the possibility to obtain the electronic properties of large molecular systems. Thus, the SHB_interactions program offers the possibility to perform such calculations for a large set of DNA and RNA structures in a relative short time, as reported elsewhere [26]. Moreover, these results show that there is a clear delimitation between H-bond overlap populations when the acceptor is an oxygen atom and those when the acceptor is a nitrogen atom. This is evidence for the capability of the overlap population to make distinction between different H-bond types, and allows comparative analysis of the results for the same type of H-bonds [26].

3. Results and discussion

The following strategy was adopted in our study:

(a) In a first step the drug was optimized (by molecular mechanics and semi-empirical AM1 methods) and conformational search was performed in order to obtain

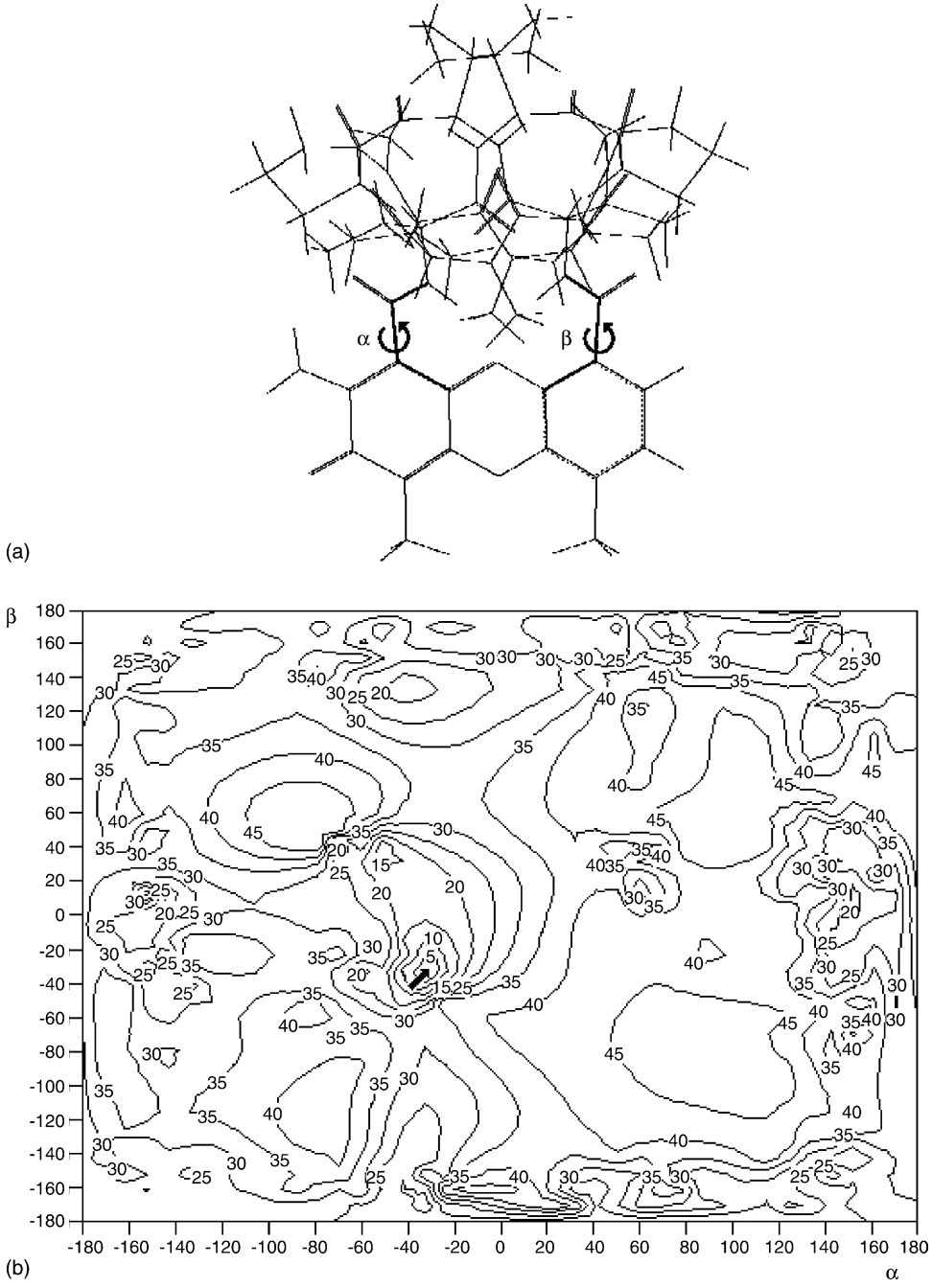

Fig. 2. (a) Rotation angles α and β in respect with the conformational search was performed. Dihedral angles are marked in bold and (b) relative energy contour plot of the actinomycin D conformers. Isoenergetical curves differ by 5 kcal/mol; the minimum energy conformer is indicated by arrow.

the most stable conformer to be used in further calculations;

(b) then the actinomycin D–dsDNA complex, with the geometry from the 1-dsc-PDB file was used as starting data in the intermolecular interactions calculations. For the complexes for which no geometrical data were available in literature, the models proposed were built and optimized with MM^+ molecular mechanics;

(c) finally, all these complexes were analyzed using SHB_interactions program, and different atom–atom interactions are outlined and discussed.

AM1 optimization in vacuo and MM^+ conformational search of the molecular structure of actinomycin D in respect with the rotation angles α and β (Fig. 2a) lead to several conformers.

The results of MM^+ conformational search are presented in Fig. 2b, and the five lowest energy conformers are listed in Table 1. The lowest energy conformer, marked by arrow in Fig. 2b, presents a geometry similar (RMS fit 0.4025 Å) to that obtained by X-ray diffraction for the crystal structure of the drug (PDB code: 1a7y) and was therefore used in further calculations. A similar result was obtained for the AM1 optimization, the RMS fit with the crystal structure being 0.1681 Å.

For the complex of the drug with dsDNA an oligonucleotide 5′-d(GAAGCTTC)-3′ was considered, and the geometry from the 1-dsc-PDB file was used without further optimization. For the complex actinomycin D–ssDNA, for which no geometrical data were available, a structure based on the hemi-intercalation model [12,13] was built. A bases sequence ATAGTT was considered, in agreement with literature data [12,13], which attest that the TAGT oligonucleotide is essential for this model. This structure was optimized using MM^+ force field from the HyperChem package.

Table 1
The relative energies (E_{rel}) of the five lowest energy conformers of actinomycin D, in respect with dihedral angles α and β

	E_{rel} (kcal/mol)	α	β
1	0	−33.8	−38.9
2	12.7	−49.9	44.1
3	15.7	−144.0	10.6
4	15.8	145.0	8.2
5	17.1	−39.2	134.2

The complexes of actinomycin D with dsDNA and, respectively, ssDNA fragments are presented in Fig. 3a and b.

The binding energies in Fig. 3 were calculated as the difference between the MM^+ energy of the complex and the sum of the energies of the molecules with the geometry frozen as in the complex. The results point out that the binding energy of the drug with ssDNA is about half from the value obtained for the actinomycin–dsDNA complex, in agreement with the hemi-intercalation model.

The interaction with the dsDNA sequence implies the intercalation of the phenoxazone moiety between the G4-C13 and C5-G12 base pairs, the pentapeptide rings being oriented to the minor groove of the DNA sequence.

In the actinomycin D–ssDNA complex, the chromophore of the drug is stacked between the A and G successive bases and the peptide chains are directed towards the neighboring bases in the sequence, leading to a bending of the single-stranded DNA fragment, as suggested by the hemi-intercalation model.

Our previous study regarding the influence of ionic strength on the interaction of actinomycin D with dsDNA indicates a major non-electrostatic contribution (90%) to the binding free energy [27]. This non-electrostatic component reflects the contribution of hydrophobic, van der Waals interactions, as well as of hydrogen bonds to the stability of the drug–dsDNA complex.

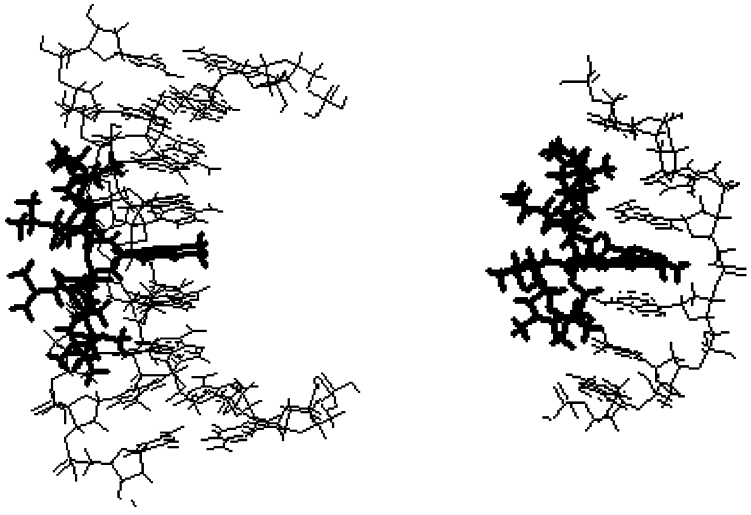

Fig. 3. (a) The complex of actinomycin D with double-stranded DNA sequence (5′-d(GAAGCTTC)-3′). (b) The complex of actinomycin D with single-stranded DNA fragment (ATAGTT).

Analysis of the different types of interactions in these complexes was performed with the program SHB_interactions [19]. In this program, the Mulliken overlap populations calculated in the frame of the Extended Hückel method are used for the evaluation of the interaction strength. The calculations performed with this program on a large number of chemical compounds (hydrocarbons, alcohols, amino acids, pyrimidinic and purinic bases) have shown that covalent bonds (bond length up to 1.6 Å) have overlap populations in the range 0.14–2.5, whereas for the intermolecular interactions ($r > 1.7$ Å) the overlap populations are at least an order of magnitude lower, i.e. in the range 0.001–0.06 [26].

In the case of the interaction of actinomycin D with double-stranded DNA, the calculated intermolecular overlap populations versus the atom–atom intermolecular distance are presented in Fig. 4. It may be observed that interactions with overlap populations up to 0.06 may be divided into three types: classical hydrogen bonds, the much controversial C–H···O(N) hydrogen bonds and other atom–atom intermolecular interactions, all of them similar in strength with hydrogen bonds in Watson–Crick base pairs G4-C13 and C5-G12, included also in Fig. 4 for the sake of comparison.

The calculated overlap populations for the specific hydrogen bonds between the bases of the double-stranded DNA and the different functional groups of the drug are presented in Table 2. Entry 2 and 3 in Table 2 correspond to the hydrogen bonds between the guanine 2-amino groups and the carbonyl oxygen atoms of the L-threonine residues of the cyclic pentapeptides, outlined also by Sobell and Jain [6,7]. It may be observed that for the same acceptor (O), a greater overlap population corresponds to shorter H···O distance. However, comparison of entry 2 and 4 shows that for similar r values a much greater overlap population is found for H···N as against H···O bonds, in agreement with previous results on a large set of DNA and RNA structures [26].

Table 2
Hydrogen bonds between actinomycin D and double-stranded DNA

DNA	Actinomycin D	r	Overlap population
N3 G A 4	H THR C 18	2.0067	0.02114
1H2 G A 4	O THR C 18	1.8493	0.02518
1H2 G B 12	O THR C 23	1.6617	0.04909
N3 G B 12	H THR C 23	1.8334	0.05380
O4* C A 5	2HN2 PXZ C 17	2.0155	0.01721
O3* G A 4	1HN2 PXZ C 17	2.8905	0.00903

Analysis of the model complex actinomycin D–ssDNA (Fig. 3b) in the frame of SHB_interactions program outlines that the overlap populations, presented in Fig. 5, are up to 0.014, i.e. smaller than for the interaction of the drug with dsDNA, in agreement with the calculated binding energy, and attesting to the weaker interaction suggested by the hemi-intercalation model.

It is interesting to note that interactions implying C–H group as potential hydrogen donor with different acceptor atoms (Figs. 4 and 5) correspond to overlap populations up to 0.025, i.e. in a range where classical hydrogen bonds and other atom–atom intermolecular interactions are also observed. Studies on small molecules based on quantum-chemical methods suggest that C–H···O interactions can be considered as weak hydrogen bonds [28,29].

There is an increasing awareness that the contribution of C–H···X(O, N) hydrogen bonds may be relevant for the stability of different biological macromolecules [30–33]. To our knowledge, the detection of these interactions as possible candidates for attractive interactions in biopolymers was restricted to geometrical criteria. We think that overlap populations can be a useful tool not only for the identification, but also for the classification according to the

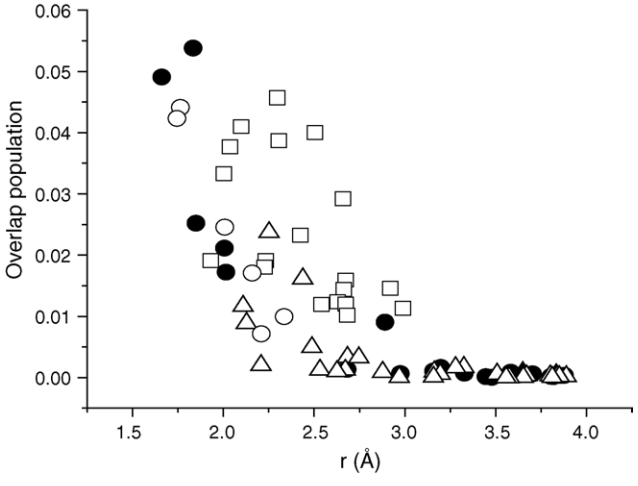

Fig. 4. The overlap populations vs. interatomic distance r, for the interaction of actinomycin D with double-stranded DNA: (●) classical hydrogen bonds; (△) C–H···O (N) hydrogen bonds; (□) other atom–atom intermolecular interactions; (○) hydrogen bonds in Watson–Crick base pairs G4-C13 and C5-G12.

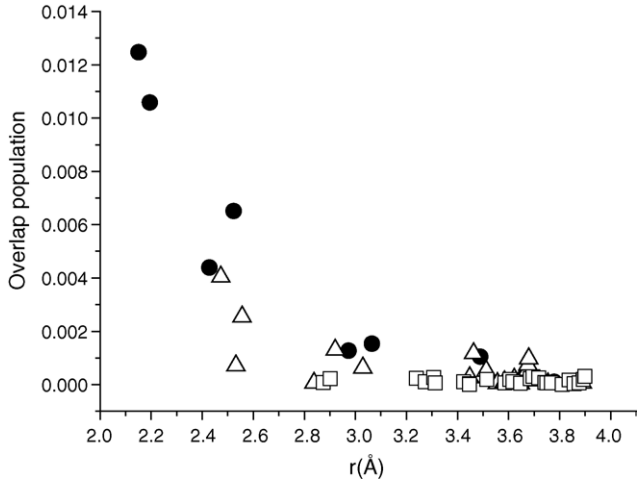

Fig. 5. The overlap populations for the interaction of actinomycin D with single-stranded DNA: (●) classical hydrogen bonds; (△) C–H···O (N) hydrogen bonds; (□) other atom–atom intermolecular interactions.

strength, and allow an estimate of their contribution to the stability of the different complexes. Thus, the contribution of the C–H···X bonds is evaluated, considering the overlap population criterion, to about 10% for dsDNA and, respectively, 26% for ssDNA.

Our theoretical results underline also that the major contribution (∼70%) to the stability of the drug–DNA complexes is due to classical H-bond interactions in the case of ssDNA and to other atom–atom interactions for dsDNA.

Use of molecular mechanics simulations corroborated with SHB_interactions program allows an insight in the nature of the non-electrostatic interactions contributing to the stability of the actinomycin D–DNA intercalation complexes. The results furnish a partial theoretical support to our experimental study based on the influence of ionic strength on the drug–DNA binding process, which evidenced a major contribution of the non-electrostatic interactions to the stability of the complexes.

Although the present approach implies in vacuo optimizations, the results allow a rationalization of the intercalative binding process (process b) of actinomycin D to both single- and double-stranded DNA. The results outline also the importance of using quantum-chemical criteria (EH overlap populations) as an estimate of the different relative contributions to the binding process of the drug to nucleic acids.

However, as outlined by previous experimental data, the drug–nucleic acids interaction is a complex process in which hydration and cooperativity effects may play a crucial role. Therefore, more studies are necessary to account for these effects and are in progress in our laboratory.

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