THEORETICAL STUDY OF CONFORMATIONAL FLEXIBILITY OF DISTAMYCIN-A ANALOG AND ITS BINDING TO DNA

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ABSTRACT

Conformational flexibility of distamycin-A analog and its binding to four pentameric sequences of DNA has been studied by conventional conformational analysis technique together with computer-based model building and dynamic energy minimization. Our results show six possible conformations of methyl pyrrole rings and a flexible chain structure. Global minima had conformational energy 2.42 kcal/mol lower than the crystallographic conformation. Considerable reduction in the binding energy was observed when full flexibility for rotation around single bonds in the drug molecule was allowed. Our final model in minor groove resembled the netropsin—DNA single crystal model. It had conformational energy 4.45 kcal/mol higher than the global minima and preference for AT containing sequences. Binding in major groove was weaker compared to the minor groove. Role of conformational flexibility of the ligand in DNA recognition is discussed in the paper.

INTRODUCTION

NON-intercalating antitumour antibiotics of netropsin-distamycin class have attracted great attention in recent years[1-6] because they serve as ideal markers for AT-rich regions of DNA[7,8]. These drugs bind to B-form of DNA in the minor groove and stop its replication by glueing both the strands[1]. Several new ligands are being synthesized which are likely to have a similar property[9-12]. The main question is: what are the physico-chemical characteristics of such sequence specific markers? Secondly, what is the role of topological factors vis-a-vis the functional groups in DNA recognition?

We report here our results on conformational flexibility and binding of distamycin-A analog with its propylaminidinium chain replaced by $CH_2-CH_2-C=N$ to DNA. This drug is chosen because of its structural data is available[13]. Another reason was the absence of physico-chemical data on its binding to DNA.

METHODOLOGY

Conformation energy calculations are carried out in the usual way with parameters discussed earlier[14]. We use three-fold symmetric torsional potential with C-C and C-N barriers respectively equal to 1.0 and 1.5 kcal/mol[15]. The effect of increasing C-C barrier to 3.5 kcal/mol[16] and C–N to 6.0 kcal/mol[17] or neglecting it totally has been also studied. Isoenergy contours for simultaneous rotations around C_3-C_6, $N_3-C_7(\alpha,\beta)$ C_9-C_{12}, N_5-C_{13} (γ,δ) and N_5-C_{13}, $C_{13}-C_{14}$ (δ,ϵ) bonds have been constructed (See figure 1 for nomenclature). Conformation of the methyl pyrrole ring is kept planar. Angles which are not rotated are kept in trans or minimum energy conformation (table 1).

Modelling of distamycin-A with $d(A)_5.d(T)_5$ (DNA 1); *d*(*TATAT*). *d*(*ATATA*), (DNA 2); *d*(*AGAGA*). *d*(*TCTCT*) (DNA 3) and $d(C)_5.d(G)_5$ (DNA 4) was attempted the same potential, starting from the absolute minimum energy conformation. This model showed very small affinity to the DNA. Hence, the functional groups were properly oriented to make hydrogen bonds with DNA bases. Modelling was done retaining the flexible chain conformation and allowing rotational flexibility around C_3-C_6 and N_1-C_7 bonds, using the procedure described earlier[18].

RESULTS AND DISCUSSION

A typical (α,β) map for dielectric permeability value of 4 is shown in figure 2 (a). Absolute minimum in this map is observed at 240°, 120° (A). Five other local minima are observed within 0.5 kcal/mol (table 1). Increasing the barriers affected the transition probability between these conformations, but did not affect the overall conformational picture. Neglecting the barriers leads to expansion of the local minima. High energy barriers were observed around $\alpha = 180°$ where the amide N,H

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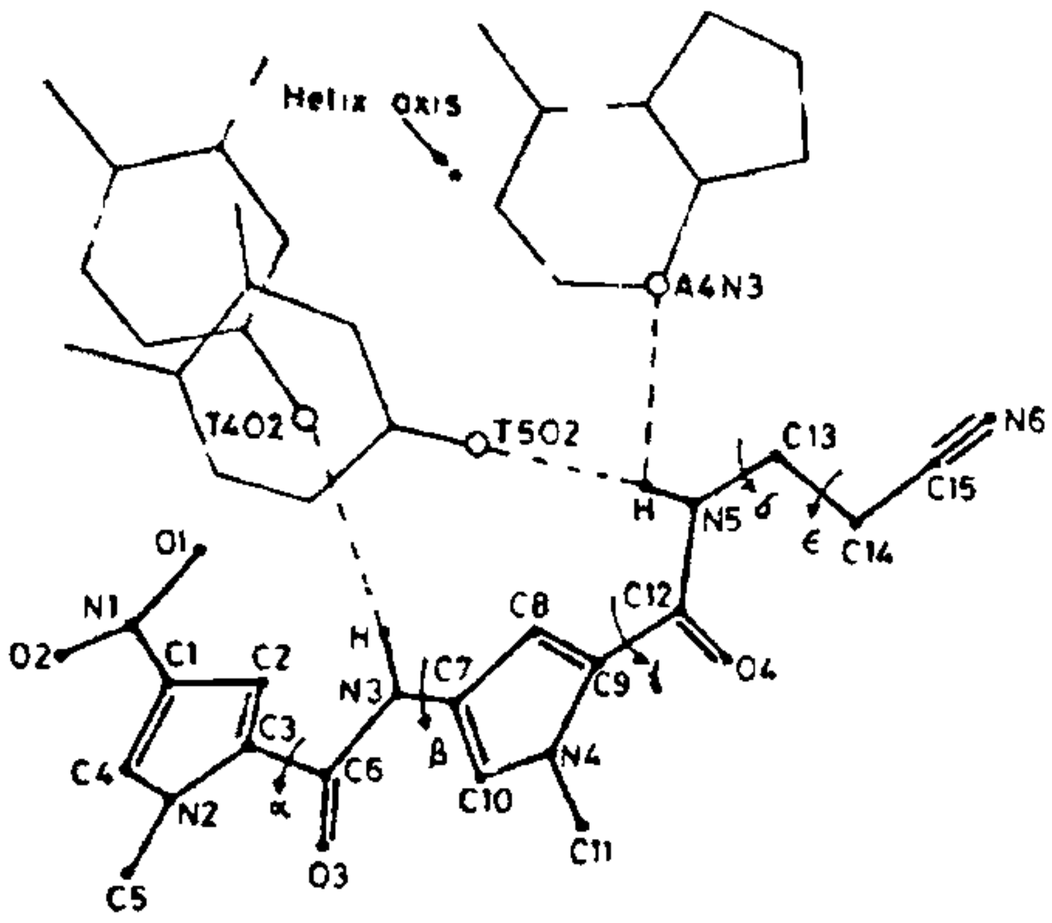

Figure 1. Top view (perpendicular to helix axis) of distamycin-A analog with $CH_2-CH_2-C\equiv N$ side chain, in the minor groove of DNA. Hydrogen bonding bases are drawn in thick line and hydrogen bonds are shown by dashed lines. Rotational angles α, β, γ, δ and ϵ are as defined in the footnotes to table 1.

comes close to the methyl group at N_2. A small barrier of 4.0 kcal/mol is observed at $\beta = 0°$.

Methylpyrrole rings are parallel in the case of conformations A and C and are inclined at 60° in the remaining conformations. Crystallographic data by Gurskaya *et al*[13] show that they are inclined at about 4° whereas Gursky *et al*[19] proposed that they should be inclined at 40–60° for binding to DNA[19]. The amide N_3H is coplanar with the pyrrole ring I in the conformations E and F and inclined at 60° in A, B, C and D conformations.

Conformational map for γ,δ rotations appeared to be quite similar to α,β map. It showed forbidden zone near δ equal to 180° due to the proximity of second methyl group (at C_{11}) with amide N_5H. Four minima were observed within a small energy interval (table 1) (δ,ϵ) rotation also showed a flexible chain structure (figure 2b). The plane of the second peptide was inclined at 60° with respect to the second pyrrole group. The same angle in the crystallographic study was 27.83°. It has energy 2.42 kcal/mol lower than the crystal conformation (table 1).

Binding of distamycin-A analog with DNA:

a. Minor groove: Binding to different DNA sequences was weak (average binding energy–EB = 17.56 kcal/mol) when distamycin was taken

Table 1 *Conformation energy calculations summary*

Con.	α	β	γ	δ	ϵ	ΔE kcal/mol	R_1-A_1	A_1-R_2	R_1-R_2
A	240	120	180	180	180	2.30	60	60	0
B	120	120	180	180	180	2.38	60	60	60
C	120	240	180	180	180	2 41	60	60	0
D	240	240	180	180	180	2 67	60	60	60
E	0	120	180	180	180	2 6	0	60	60
F	0	240	180	180	180	2.74	0	60	60
X_1	240	120	120	120	180	1.42	60	60	0
X_2	240	120	240	240	180	1.40	60	60	0
X_3	240	120	240	120	180	1.11	60	60	0
X_4	240	120	120	240	180	1 03	60	60	0
Y_1	240	120	120	120	320	0	60	60	0
Cryst	350	190	27.83	76.25	180	2.42	4	4	4
Model	336	182	349	344	180	4.45	20.49	2.60	18.27

Torsional angles α and β are defined as $C_2-C_3-C_6-N_3$, $C_6-N_3-C_7-C_8$, γ,δ and ϵ are defined as $C_8-C_9-C_{12}-N_5$; $C_{12}-N_5-C_{13}-C_{14}$ and $N_5-C_{13}-C_{14}-C_{15}$ respectively R_1-A_1, A_1-R_2 and R_1-R_2 denote the angles between rings and amides. ΔE represents change in the conformational energy with respect to global minima.

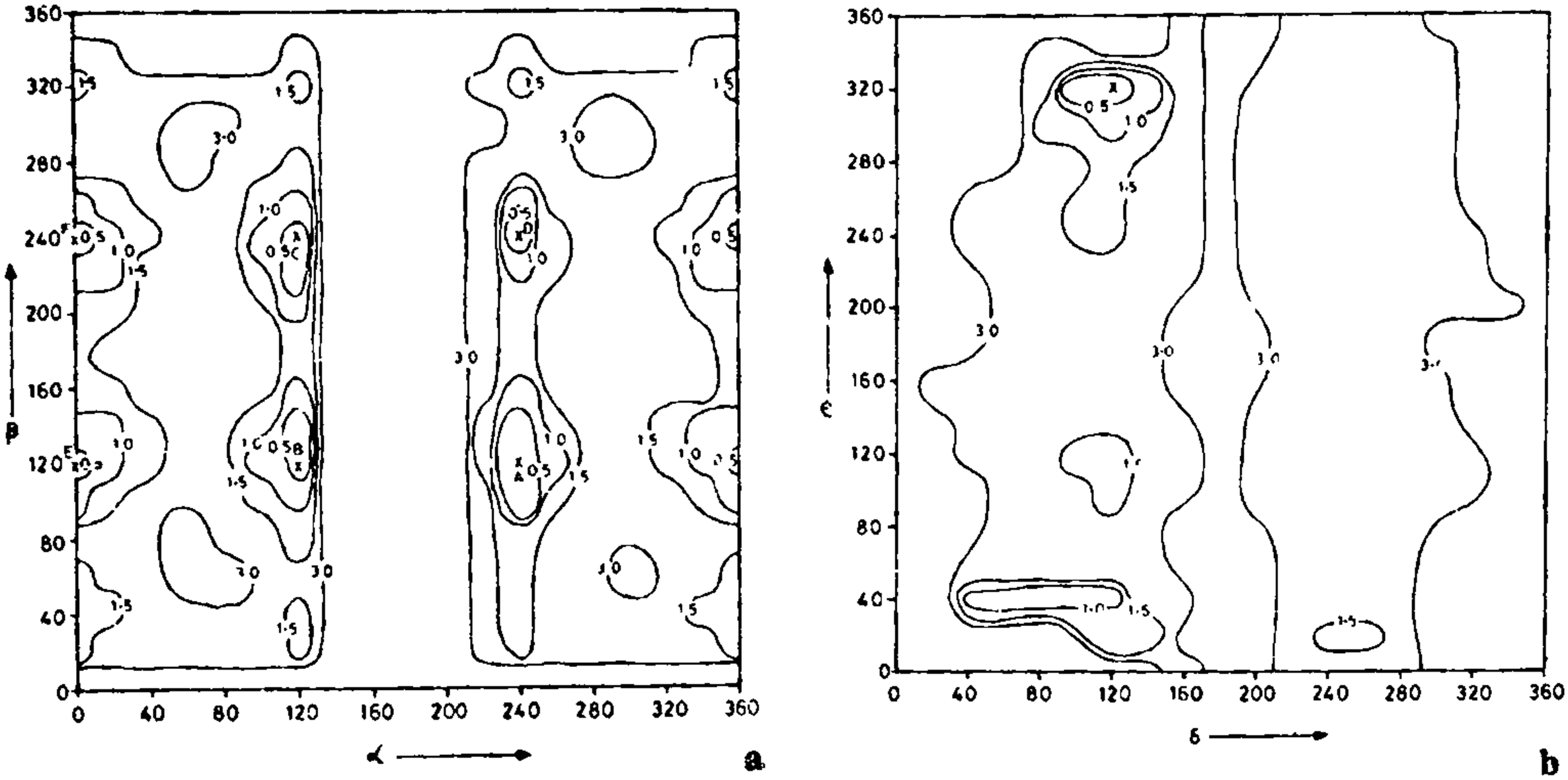

Figure 2. Conformational maps for rotations around (a) C_3–C_6 (α) and N_3C_7 (β) and (b) N_5–C_{13} (δ) and C_{13}–C_{14} (ϵ) in 20° interval. Isoenergy contours are drawn for energies 0.5, 1.0, 1.5 and 3.0 kcal/mol with respect to the absolute minimum in the map, at dielectric constant=4.0.

in its minimum energy conformation (table 2). It also showed very small sequence specificity (ΔE = 0.85 kcal/mol). Considerable reduction in the binding energy was observed when flexibility for rotation around various single bonds was allowed. Our final model resembled netropsin DNA single crystal model by Kopka *et al*[4] and had bifurcated hydrogen bonds between amides N_3H and N_5H and electron acceptor groups of adjacent base pairs on opposite strands (figure 1). It had preference for AT-rich sequence (ΔE=4.36 kcal/mol). Sequence specificity arose from electrostatic as well as hydrogen bonding interactions which showed maximum variation of 4.64 and 3.58 kcal/mol respectively.

b. Major groove: Binding in major groove was weaker compared to minor groove (table 2). This reduction in binding was mainly because of increase in non-bonded contributions between the DNA backbone and the rings, as the drug is loosely fitted in the major groove. The electrostatic energy of the rings also increased with bases and backbone. The drug is slightly asymmetrically fitted in the case of AT sequence. Energy partitioning with the two

Table 2 *Conformational and Interaction Energy (kcal/mol) of distamycin-A analong with different base sequences of DNA*

DNA sequence	Groove	Conformation of the drug	Relative conformation energy	Interaction energy (EB)	Total Energy
DNA 1	Minor	*A*	0.0	−18.21	−18.21
DNA 4	Minor	*A*	0.53	−17.36	−16.83
DNA 1	Minor	Model	4.45	−73.33	−68.88
DNA 2	Minor	Model	4.37	−72.48	−68.11
DNA 3	Minor	Model	4.25	−70.13	−65.87
DNA 4	Minor	Model	4.31	−68.97	−64.66
DNA 1	Major	Model	4.30	−46.817	−42.517
DNA 4	Major	Model	4.20	−38.044	−33.844

strands has value 38:62 for AT sequence. In the case of GC sequence there is more or less symmetric energy partioning (47:53) of energy. We observe preference for AT sequence as in the case of a minor groove. Zakrzewska *et al*[6] observed larger binding in major groove for netropsin for GC sequence compared to AT sequence because of positively charged tails in netropsin. They give large attraction with DNA backbone. On the contrary we have observed repulsion with the tails.

CONCLUSION

Our results show that the nature of tails as well as their flexibility is important for sequence preference. Groove preference is mainly because of the specific size of the drug. Hydrogen bonding, although does not contribute much towards stabilization of the drug DNA interaction, assists in "hinging" the drug in the groove. Inability to make proper hydrogen bonds in the major groove is responsible for the preference of minor groove in these ligands.

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