Volume 215, number 1, 95–99 FEB 04631 May 1987

Theoretical study on binding of Hoechst 33258 with oligonucleotides

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Received 4 February 1987

Computer modelling with an energy minimization procedure is used here to obtain stereochemical and energetic details for complexes of the dye Hoechst 33258 with different oligonucleotide sequences. An optimised model of the dye with d(A)5·d(T)5 is in conformity with previous proposed models. It has bifurcated hydrogen bonds between N_2H and N_4H of benzimidazole rings with N_3 of adenine and O_2 of thymine. Relative binding energies with different oligonucleotides show preference for AT containing sequences, with an intermediate affinity between that for netropsin and distamycin-2. Reduced binding is observed at high ionic concentration. The benzimidazole rings are twisted with respect to the phenol ring in the optimal model. This gives desired curvature to the molecule which is stabilised by intermolecular forces.

Computer simulation; DNA-dye interaction; Hoechst 33258

1. INTRODUCTION

Bis-benzimidazole derivative Hoechst 33258 has been used as an effective DNA binding fluorochrome in chromosomal binding patterns [1,2]. Spectroscopic studies by Latt and Wohlleb [3] showed that it binds preferably to AT containing sequences. This leads to a red shift of the UV absorption spectrum and increases the DNA melting temperature [4–6]. Two external binding modes were proposed by Bontemps et al. [4] while attachment to the major groove was suggested by Latt and Wohlleb [3] and Latt et al. [6] on the basis of fluorescence spectroscopic studies. CD spectroscopic studies by Zasedatelev et al. [7–9], as well as ^{125}I decay studies by Martin and Holmes [10] indicated minor groove binding of the dye. They proposed that NH groups of two benzimidazole rings form bridging hydrogen bonds with N_3 of adenine and O_2 of thymine and cover about a four base pair region. This view is later supported by foot-printing studies [11,12].

The dye has recently been crystallised with the CGCGAATTCGCG dodecamer [13]. Their preliminary results confirm minor groove binding of the dye analogous to netropsin. However, detailed models with different oligonucleotides and quantitative evaluation of intermolecular forces for them are still absent in the literature. We present here energy optimised models for the interaction of the dye with different oligonucleotic sequences. This technique has gained popularity in the recent past for the prediction of geometries of complexes of DNA with oligopeptides and non-intercalating antitumour antibiotics [14–21]. It is therefore desirable to elucidate the stereochemical and energetics details of these models.

We have also considered here the effect of change in dielectric permeability and counterion distribution upon binding of the dye with oligonucleotides.

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

2. METHODOLOGY

An empirical potential energy function used for conformation and interaction energy calculations consists of Lennard-Jones attractive and repulsive, electrostatic monopole, dipole induced dipole (polarisation), hydrogen bonding and torsional terms. Except for partial atomic charges and torsional potential, all other parameters were taken from Momany et al. [22]. Partial atomic charges were taken from literature on oligonucleotides [23]. They were computed for the dye molecule on the basis of the CNDO/ON method [24]. In agreement with the conventional structural formula [11,12] unit positive charge was assumed on the piperazine ring while benzimidazole rings were considered to be neutral for these calculations. The pK value for benzimidazole being 5.48 [25], one does not also expect much positive charge on this ring at physiological pH.

The dye molecule is structurally quite rigid because of three unsaturated rings (one phenol and two benzimidazoles). It has limited flexibility for rotations around C_4-C_7 (θ), C_{11}-C_{14} (ϕ) and C_{18}-N_5 (ψ) bonds (see fig.1 for nomenclature). The piperazine ring has also some flexibility. We considered a two-fold symmetric barrier for rotations around C_4-C_7 and C_{11}-C_{14} bonds. Its value was taken as equal to 5.5 kcal/mol so as to allow for the partial double bond nature of this bond [26]. For rotation around C_{18}-N_5, a combination of two-fold (V_2) and three-fold (V_3) barriers was used. V_3 was large (14.0 kcal/mol) whereas V_2 was taken to equal 9.6 kcal/mol [26]. This was done to ensure the planar tendency of the sp^2 carbon as well as to keep the asymmetric center at N_5.

The effect of counterion current distribution was incorporated by introducing a Debye Hückel screening factor for electrostatic and dipole-induced dipole interaction terms [27]. The optimal conformation of isolated dye molecules was first obtained allowing complete flexibility for rotation around C_4-C_7, C_{11}-C_{14} and C_{18}-N_5 bonds, and assuming planar conformation for the piperazine ring. This conformation was taken as the starting geometry for model building. NH groups of the benzimidazole rings were then oriented towards hydrogen acceptors in the minor groove of oligonucleotides d(A)5·d(T)5, d(ATATA)2, d(ACACA)2 and d(C)5·d(G)5. Geometries of the complexes were optimised allowing conformational as well as motional freedom for the dye. The conformation of oligonucleotide was kept fixed in Arnott's B form [28]. Details of this procedure are described elsewhere [18,21].

The concentration of the counterions (mono- as well as divalent) was varied from 0.2 to 1 M. Dielectric permeability value ϵ was allowed to change between 4 and 20.

3. RESULTS AND DISCUSSION

3.1. *Geometry*

The isolated dye molecule assumes a planar conformation because of the partial double bonded nature of C_4-C_7, C_{11}-C_{14} and C_{18}-N_5 bonds and the use of two-fold symmetric barriers (table 1). In models with oligonucleotides the piperazine ring is coplanar with an adjacent benzimidazole ring, whereas other benzimidazole and phenol rings are twisted with respect to each other. The angle between phenol and benzimidazole ring is significantly different in the case of different models (table 1). Twisting of phenol and benzimidazole rings gives curvature to the molecule and brings N_2H and N_4H groups into a hydrogen bonding position.

The dye molecule has an end-to-end distance of approx. 20 Å and covers a four base pair region (fig.2). N_2H and N_4H form bridging hydrogen bonds with adjacent base pairs (fig.1 and table 2) in the case of d(A)5·d(T)5 and d(ATATA)2. This model is in conformity with the earlier proposed models [7–13]. The dye molecule had to be pulled away from the DNA helix axis so as to avoid short contact with NH_2 of G2 and G4 in the case of d(ACACA)2 and G2, G3, G4, G5 in d(C)5·d(G)5. This leads to longer and weaker hydrogen bonds in these two cases.

3.2. *Energetics*

Twisting of the benzimidazole and phenol rings leads to increased conformation energy (table 1). This increase is compensated by intermolecular interactions (table 3).

In all the four models we observe a larger contribution to the interaction energy by the 5′-strand of DNA because the dye molecule is shifted more towards it. The ratio E_{non}/E_{el+pol} for d(A)5·d(T)5 was equal to 0.95 for the dye. The value in the case of netropsin and distamycin-2 was 0.14 and 0.35,

Table 1

Comparison of interplanar and dihedral angles of different models for Hoechst 33258

Models	Angles between rings			Dihedral angles			
	A–B	A–C	A–D	θ	ϕ	ψ	ΔE (kcal/mol)
1. Minimum energy conformation	0.0	0.0	0.0	0.0	180.0	0.0	0.0
Optimised models							
2. With d(A)5·d(T)5	40.08	22.97	22.89	139.9	−158.1	0.0	34.6
3. With d(ATATA)2	41.97	22.90	22.80	138.0	−158.1	0.0	34.6
4. With d(ACACA)2	24.98	45.34	45.94	24.98	−158.0	0.0	33.6
5. With d(C)5·d(G)5	24.90	45.00	45.95	24.9	−157.0	0.0	33.6

Here A, B, C, D refer to phenol, benzimidazole 1, benzimidazole 2 and piperazine rings (fig.1). Dihedral angles θ, ϕ, ψ are respectively C_3-C_4-C_7-N_1, C_{10}-C_{11}-C_{14}-N_4, C_{17}-C_{18}-N_5-C_{21}. All angles are in degrees. E is relative conformational energy

respectively [18]. A larger non-bonded contribution in the case of the dye was caused by bulky unsaturated rings. Considerable reduction in this ratio is observed for d(ACACA)2 and d(C)5·d(G)5 because of steric hindrance. Thus steric factors played an important role in recognition of DNA by Hoechst 332258. Contrary to this, changes in a nonbonded contribution in netropsin and distamycin-2 were much smaller for different base sequences [18]. This was because there was

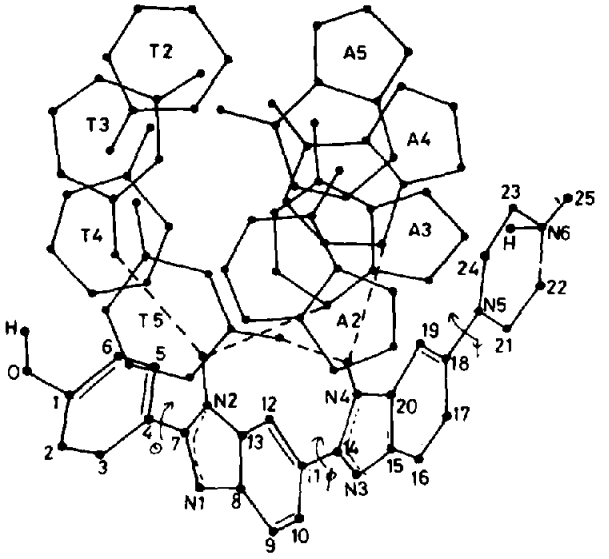

Fig.1. Top view (perpendicular to helical axis of DNA) of the dye molecule along with bases involved in hydrogen bonding for the model of dye with DNA1. Here A_2, A_3, A_4, A_5, T_2, T_3, T_4, T_5 refer to adenine and thymine bases on the 5′- and 3′-strands. The figure also shows hydrogen bonds by dotted lines. The torsional angles (θ, ϕ, ψ) are defined in table 1.

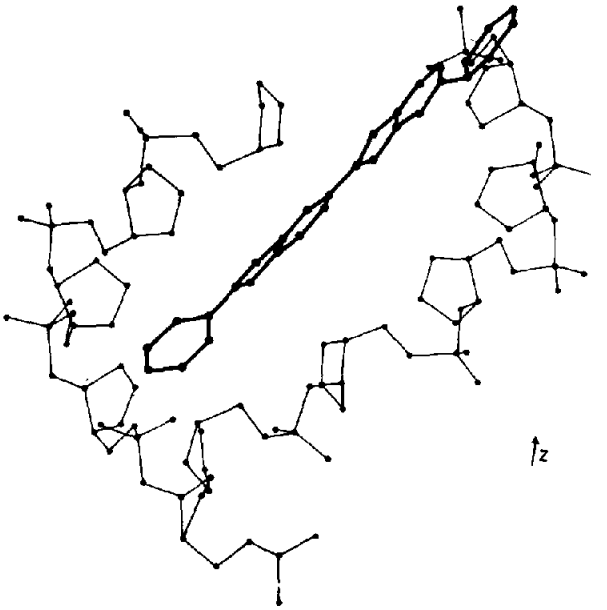

Fig.2. Front view (along the helix axis) of the dye molecule with a DNA backbone in the case of its model with DNA 1. The dye molecule is seen to occupy a four base pair region.

Table 2

List of hydrogen bonds of optimised models with different oligonucleotides

Oligonucleotide sequence	Hoechst atom	DNA base	RHB in Å
1. d(A)5·d(T)5	N_2H	A_3	3.23
	N_2H	T_3	3.03
	N_4H	A_4	2.57
	N_4H	T_5	2.77
2. d(ATATA)2	N_2H	A_3	2.93
		T_3	3.37
	N_4H	A_4	2.57
		T_4	3.88
		A_5	2.85
3. d(ACACA)2	RHB above 4 Å		
4. d(C)5·d(G)5	RHB above 4 Å		

In adenine the hydrogen bonding atom is N_3 and in thymine the hydrogen bonding atom is O_2. The numbers indicate the position of bases from 5′-end

enough room for them in a form of DNA and steric forces played a lesser role.

Specificity Δ defined as the ratio of maximum difference in the binding energy to average binding energy has a value 0.51 for the dye and is larger than in netropsin and distamycin-2 (Δ = 0.19 and 0.11 for netropsin and distamycin-2, respectively) [18]. This enhancement in the specificity was due to larger nonbonded contributions.

Thus, all the three types of forces, nonbonded, electrostatic (interaction of piperazine and benzimidazole with DNA bases) and hydrogen bonding, contribute towards sequence specificity. Electrostatic interaction of the piperazine with DNA backbone plays a great role in stabilization of the dye DNA complex but did not affect sequence specificity.

Relative affinity of the dye with d(A)5·d(T)5, as estimated from interaction energy, was intermediate between that for netropsin and distamycin-2. This is contrary to results by Zasedatelev et al. [7–9] who observed a larger K value for the dye than for netropsin. The reason for the latter was that the compound used by them had positively charged piperazine as well as benzimidazole rings. The latter leads to a 3-fold increase in the electrostatic monopole and dipole-induced dipole contributions. This would naturally give stronger binding compared to netropsin. With a unit positive charge its binding is expected to be of the same order as distamycin-2. We observed a slightly stronger value (ΔE = −11.92 kcal/mol) because of larger Van der Waals (attractive-repulsive) contributions in the case of the dye.

Increasing charge of the ion, concentration, and also the dielectric permeability constant leads to increase in the binding energy of the dye with DNA and reduction in the binding affinity to DNA. Some residual binding is observed in high salt conditions because of non-electrostatic forces (table 3)

Table 3

Partitioning of interaction energy of Hoechst 33258 for different DNA sequences

DNA sequence	Strand I	Strand II	E_{non}	E_{el+pol}	E_{hydg}	E_{total}
d(A)5·d(T)5	−103.42	−76.04	−83.93	−87.48	−8.05	−199.46
d(ATATA)2	−100.46	−79.85	−83.84	−88.00	−7.62	−180.31
d(ACACA)2	−73.09	−49.41	−41.06	−77.52	−3.92	−122.50
d(C)5·d(G)5	−64.34	−39.67	−36.02	−64.67	−3.32	−104.01

Strand I, interaction energy with 5′ strand; strand II, interaction energy with 3′ strand; E_{non}, sum of Lennard-Jones attractive and repulsive contribution; E_{el+pol}, sum of electrostatic monopole and dipole induced dipole contributions; E_{hydg}, hydrogen bonding energy; E_{total}, total interaction energy. All energies are in kcal/mol

which explains why the dA-dT specific complex of the dye is stable at 4 M NaCl or 6 M LiCl [7,9].

4. CONCLUSION

The two factors most crucial for minor groove binding of A-T specific ligands are: (i) the presence of hydrogen bond donors; (ii) conformational flexibility which can permit isohelical structure formation for the ligand. All the three types of forces, non-bonded, electrostatic and hydrogen bonding, contribute towards specificity. An environmental effect on recognition depends on the relative contributions of these terms. Structural compatibility of the ligand, although it helps its interaction with DNA, eventually plays a smaller role in sequence specificity. This is the reason why structurally dissimilar molecules can exhibit similar binding. In contrast to this, structurally similar molecules may exhibit dissimilar binding.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Science and Technology for financial assistance and awarding fellowships to M., A.K.R. and K.G. We are also thankful to the Council of Scientific and Industrial Research for awarding a post-doctoral fellowship to A.K.

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