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On the origin of the decrease in stability of the DNA hairpin d(GCGAAGC) on complexation with aromatic drugs

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Abstract

Molecular dynamics simulations of drug–DNA complexes have been carried out in order to explain the experimentally observed decrease in thermal stability of the DNA hairpin d(GCGAAGC) on binding the aromatic drug molecules, daunomycin, ethidium bromide, novantrone and proflavine. This complexation behavior is in contrast to the stabilizing effect of the same aromatic drug molecules on DNA duplexes. Analysis of the energy parameters and the hydration properties of the complexes shows that the main factor correlating with the decrease in melting temperatures of the drug–hairpin complexes is the number of water bridges, with a reduction of at least 40% on ligand binding.

Keywords: d(GCGAAGC); Aromatic ligand; Water bridges; Thermal stability; Molecular dynamics

1. Introduction

DNA hairpins play a very important role in replication, transcription, recombination and other biological processes in cells [1,2]. The biologically important DNA hairpin, d(GCGAAGC) has been found in the initiation regions of phage *φX*174 [3], virus HSV-1 [4] and *E. coli* bacteria [5] replication. The d(GCGAAGC) hairpin is very stable; it has an extremely high melting temperature (T_m=76.5 °C in 0.1 M NaCl [6]) and exhibits great resistance to the action of nucleases [7]. A possible link between the biological function of the d(GCGAAGC) hairpin and its great thermal stability poses the question of how different physical factors might influence its stability in physiological solution. One of the important factors is the binding of aromatic drugs, whose biologico-medical action is known to be triggered via complexation with cellular DNA [8].

It has been found previously [9–11] that binding of DNA intercalators to the GC stem of d(GCGAAGC) leads to a decrease in the melting temperature T_m of the complex. The estimated values of T_m are shown in Table 1 for complexation with ethidium bromide (EB) [9], daunomycin (DAU) [10], proflavine (PF) [10] and novantrone (NOV) [11]. This behavior is opposite to the known increase in the melting temperature of regular DNA duplexes on complexation with the same ligands [12–15]. Such a difference in behavior has not been explained to date. In order to provide further understanding of the observed decrease in the stability of the d(GCGAAGC) hairpin on complexation with aromatic drugs (EB, DAU, NOV, PF), a detailed molecular dynamics (MD) simulation has been made for the intercalated complexes of the drugs with both the DNA hairpin d(GCGAAGC) and a regular DNA duplex.

2. Materials and methods

MD simulation of EB/DAU/PF/NOV complexes with d(GCGAAGC) has been performed using X-PLOR software (version 3.1 [16]) with the CHARMM27 force field incorporated. Spatial structures of the hairpin and drugs were taken from the PDB database [17]. Initial structures of the intercalated complexes were built up using sets of intermolecular NOE cross-peaks [9–11] and structural information on the binding of these ligands with DNA duplexes [18,19]. In particular, DAU/EB was inserted into the d

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Table 1
Calculated parameters of the hairpin structure d(GCGAAGC) and its complexes with aromatic ligands

Parameter	Hairpin	Hairpin+DAU	Hairpin+EB	Hairpin+NOV	Hairpin+PF	Correlation with T_m
Melting temperature [a]						
T_m (°C)	76.5 [6]	48.3 [10]	58.9 [9]	60.7 [11]	52.2 [10]	
Magnitudes of the atom–atom interaction energies (kcal/mol) [b]						
Inside the hairpin	−(612±11)	−(572±16)	−(552±10)	−(571±11)	−(570±12)	0.73
Overall hydration [b]						
Hydration index	65.2±3.0	55.2±2.5	65.2±2.8	64.3±2.8	63.1±3.1	0.67
Intramolecular water bridges						
Number of water bridges	10	3	6	5	5	0.96

[a] The melting temperatures have been calculated from the temperature dependence of the concentration of the hairpin form in solution in the point of equal proportion of the hairpin and the monomer.
[b] Averaged during the final 40 ps of MD.

(G1pC2) site of the hairpin stem from the side of minor groove, and NOV/PF molecules were inserted from the major groove side. The aqueous environment was modeled by a cubic box with a side length of 35 Å filled with TIP3P water molecules. Neutralization of the phosphate negative charges was provided by six Na^+ ions. MD simulations were carried out with a VERLET algorithm [20] employed at a constant temperature 298 K. The evolution time for each system was 2 ns with a time-step of 2 fs using a SHAKE procedure [21]. The overall hydration of the deoxyheptanucleotide was computed as an averaged number of water molecules forming hydrogen bonds with hydrophilic atoms of DNA for the final 40 ps of MD. Hydrogen bonds and water bridges were determined using published criteria [22].

3. Results and discussion

3.1. Analysis of drug–hairpin complexes

The total energy of the calculated structures of the unperturbed hairpin and its complexes with the ligands are summarized in Table 1 and a detailed parsing of the interaction energies into different terms is provided in Table S1. One might expect that inter- and intramolecular atom–atom interactions would influence the stability of the complexes of aromatic ligands with the DNA hairpin, though that was not the case for modeling of DNA mini-hairpin molecules with various loop sequences [23]. Analysis of the data in Table 1 shows that there are only relatively small differences in the interaction energies for the set of the drug–hairpin complexes investigated and that there are no statistically meaningful correlations between the energy parameters and the set of melting temperatures (correlation coefficient about 0.73). Hence the inter- and intramolecular atom–atom interactions in the drug–hairpin complexes are unlikely to be the principal factors determining the decrease in thermal stability of d(GCGAAGC) on binding with aromatic ligands.

Another factor which might be responsible for the stability of the secondary structures of nucleic acids is interaction with the aqueous environment [24]. The calculated overall hydration index of d(GCGAAGC) in its complexed and uncomplexed form is summarized in Table 1 and the detailed information on the hydration properties is given in Table S2. It is seen from

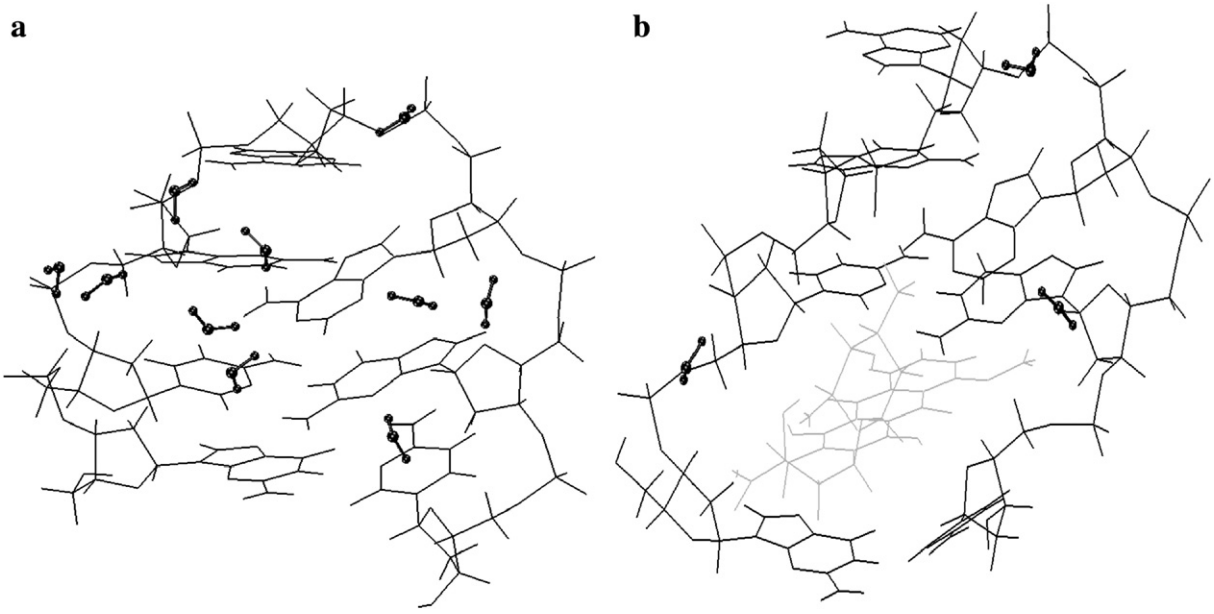

Fig. 1. Visualization of the calculated intramolecular water bridges of the hairpin d(GCGAAGC) (a) and its complex with daunomycin (b).

Table 2
Calculated parameters of DNA duplex d(GTCGAC) and its complexes with aromatic ligands

Parameter	Duplex	Duplex +DAU	Duplex +EB	Duplex +NOV	Duplex +PF
Overall hydration [a]					
Hydration index	96.8±3.7	94.0±3.7	94.0±3.7	94.6±3.9	96.1±3.8
Intramolecular water bridges					
Number of water bridges	16	13	14	14	15

[a] Averaged during the final 40 ps of MD.

Table 1 that the overall hydration profile of the hairpin on complexation with three of the aromatic drugs (EB, NOV, PF) is essentially unchanged, which means that this factor is also unable to explain the changes in melting temperatures (correlation coefficient is 0.67). The hydration index of the DAU–hairpin complex (55) is significantly lower than for the hairpin (65), which might be expected because of the known removal of water molecules from the minor groove of DNA due to the bulky amino-sugar ring of the antibiotic, when intercalating into the duplex [13].

The interactions of the drug–hairpin complexes with the aqueous environment have been investigated in more detail, in order to elucidate the key factors in determining the thermal stability of complexes. Few reports are available in literature stating that intramolecular water bridges is an important contributor to the stability of secondary structures of nucleic acids [25,26]. The calculated number of water bridges present in the unperturbed hairpin and drug–hairpin complexes is summarized in Table 1 and visualized in Fig. 1. Further detail is provided in Table S3. It is seen from Table 1 that on intercalation of the ligands with the hairpin there is a decrease in the number of water bridges by a factor of about two for EB, PF and NOV and by about three for DAU (see also Fig. 1). As the number of water bridges disrupted on binding of the aromatic ligands correlates reasonably well with the decrease in the melting temperatures (correlation coefficient of 0.96, see Table 1), it is suggested that the decrease in the number of water bridges may explain the decrease in thermal stability of the d(GCGAAGC) hairpin when complexed with aromatic drugs. This can only be correct if disruption of water bridges is a specific property of the d(GCGAAGC) hairpin and is not observed for complexation of the same ligands (DAU/EB/NOV/PF) with regular DNA duplexes for which T_m, in contrast, increases and does not depend on the nucleotide sequence [12–15].

3.2. Analysis of the drug–duplex complexes

The self-complementary hexamer sequence $d(GTCGAC)_2$ having a central CpG site to complex the ligand has been selected as a suitable model sequence to investigate the complexation of aromatic drugs with regular DNA duplexes. It has been demonstrated both theoretically and experimentally by various authors [12,18,27,28] that all the ligands studied (DAU/EB/NOV/PF) exert sequence specificity for a d(CpG) step of DNA. In addition, it has been found that DAU complexes more specifically to the triplet sequence d(CGA) with insertion of the chromophore into the d(CpG) site [27] so that a self-complementary d(TCGA) sequence forms the minimum requirement to study the complexation of the set of ligands with DNA. Finally, in order to increase the conformational stability of the model duplex, it is reasonable to flank that tetramer from both sides with G:C pairs to give the hexamer $d(GTCGAC)_2$ as a model duplex for detailed MD simulations.

MD simulations for the duplex form of $d(GTCGAC)_2$ and its complexes with the ligands were carried out using procedures similar to those used to study the heptamer hairpin d(GCGAAGC). The same hydration parameters for the drug–DNA duplex as determined for the DNA hairpin are summarized in Table 2 and further detail on the results of calculations of the interaction energies, hydration indices and intramolecular water bridges is provided in Tables S4, S5 and S6, respectively. It is found that the relative changes in the hydration profiles of drug–DNA duplexes are small compared to those calculated for the hairpin. More importantly, the number of water bridges stabilizing the secondary structure does not change significantly on binding of the ligands to the DNA duplex (Table 2), whereas the ligand binding to the hairpin structure results in disruption of at least 40% of water bridges (Table 1). It is concluded that the number of water bridges is a key factor in determining the decrease in thermal stability of the d(GCGAAGC) drug–hairpin complexes in solution.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bpc.2007.05.005.

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