Cooperativity in Drug−DNA Recognition: A Molecular Dynamics Study

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Abstract: NMR studies have shown that the minor groove-binding ligand Hoechst 33258 binds to the two T_4/A_4 tracts within the duplex d(CTTTTCGAAAAG)$_2$ in a highly cooperative manner, such that in titration experiments no intermediate 1:1 complex can be detected. The NMR-derived structures of the free DNA and the 2:1 complex have been obtained, but can shed little light on what the origins of this cooperativity may be. Here we present the results of a series of molecular dynamics simulations on the free DNA, the 1:1 complex, and the 2:1 complex, which have been designed to enable us to calculate thermodynamic parameters associated with the molecular recognition events. The results of the molecular dynamics studies confirm that structural factors alone cannot explain the cooperativity observed, indeed when enthalpic and hydration factors are looked at in isolation, the recognition process is predicted to be slightly anticooperative. However, when changes in configurational entropy are taken into account as well, the overall free energy differences are such that the calculated cooperativity is in good agreement with that observed experimentally. The results indicate the power of molecular dynamics methods to provide reasonable explanations for phenomena that are difficult to explain on the basis of static models alone, and provide a nice example of the concept of "allostery without conformational change".

Introduction

The regulation of transcription is frequently mediated through specific interactions between complex regulatory assemblies of proteins and an array of DNA sites that are often separated by significant distances.[1] A ubiquitous feature of these regulatory complexes is that they are assembled highly cooperatively in order to enhance binding affinity, sequence selectivity and sensitivity to protein concentration.[2] Homeodomain DNA binding proteins,[3−6] for example, bind as a dimer to the palindromic DNA sequence TAATCTGATTA, composed of two inverted TAAT motifs. Protein−protein interactions are evident in the complex; however changes in DNA conformation (a 21° bend) are also essential for the highly cooperative dimer−DNA interaction.[7,8] Studies of the interaction of a number of homeodomain monomers show that they also produce significant conformational changes in the DNA, presenting strong evidence that cooperative binding is mediated by DNA conformational changes brought about by an initial binding event that enhances the affinity for the second site.[9]

It is also becoming clear that cooperativity can operate in sequence-selective drug−DNA recognition. The DNA bis-intercalating anti-tumor antibiotic echinomycin binds preferentially to CpG sites; NMR and footprinting analysis of the interaction of the drug with the sequences ACGTACGT and ACGTATACGT shows that drug molecules bind cooperatively to the two CpG sites. In contrast, cooperative interactions are disrupted by the sequence TCGATCGA, demonstrating that sequence specific effects are responsible for mediating information transfer between sites.[10−12]

The origins of cooperativity in protein−DNA and drug−DNA complexes have generally become evident where structure determination has been performed. Typically close contacts are observed in the 2:1 complex between the two ligand molecules. Thus the binding of the second ligand to the 1:1 complex is associated with the formation of a greater number of favorable interactions than binding of the first ligand to the free DNA. In the case of echinomycin, it is unlikely that cooperativity is mediated by direct contact between drug molecules, but that drug-induced conformational changes at one site are propagated to the other, through effects on helical twist (helix unwinding) and minor groove width. In contrast, the oligopeptide antibiotic distamycin has demonstrated side-by-side antiparallel binding to the DNA minor groove, with favorable $\pi-\pi$ interactions

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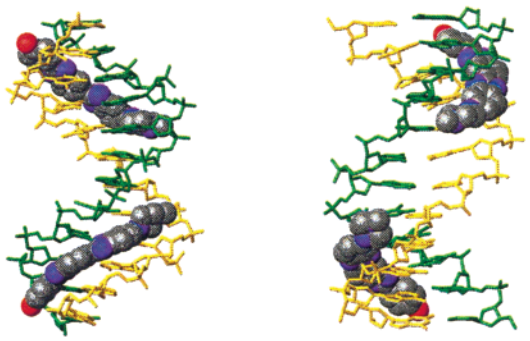

Figure 1. NMR-derived structure of the 2:1 complex between Hoechst 33258 and the DNA duplex d(CTTTTCGAAAAG)$_2$.[20] There is no direct contact between the two drug molecules.

Scheme 1. Equilibria Involved in the Formation of the 2:1 DNA/Ligand Complex and Definition of K_{coop}

$$D + 2L \underset{}{\overset{K_1}{\rightleftharpoons}} DL + L \overset{K_2}{\rightleftharpoons} DL_2$$

free DNA 1:1 complex 2:1 complex

$$K_{co\text{-}op} = \frac{K_2}{K_1}$$

stabilizing the drug dimer.[13,14] The 2:1 binding mode has been shown to be highly cooperative in the case of binding to AT-rich sequences of DNA that do not have an intrinsically narrow minor groove (such as TATAT and AAGTT), and where there is poor binding complementarity in the 1:1 complex.[15−18] In the 2:1 complex the groove width increases further to accommodate the drug dimer, requiring sufficient DNA flexibility to optimize van der Waals interactions between the drug and the walls of the groove. In this case, there is a clear shape complementarity requirement for cooperative binding that can be rationalized on the basis of a direct interaction between two bound ligand molecules.

Previously, we have reported a structural analysis of the DNA dodecamer duplex d(CTTTTGCAAAAG)$_2$ that contains two A-tracts which are preferential binding sites for minor-groove binding ligands.[19] When the titration of this dodecamer with Hoechst 33258 (H33258) is followed by NMR, signals for the free DNA are replaced by those of the 2:1 drug:DNA complex, without detection of any intermediate 1:1 complex. In terms of the equilibria involved (Scheme 1), and the practical limits of NMR sensitivity, we have estimated a lower limit on the cooperativity index, K_{coop}, of approximately 1000, which equates to a $\Delta\Delta G$ for the two binding events of −4 kcal/mol.

Interestingly, the NMR-derived structures of the free DNA and 2:1 complex do not, in this case, give us any insight into the origins of cooperativity in this system.[20] The structure of the complex (Figure 1) shows that the ligands are not in contact and are oriented in the two A-tracts in such a way that their positively charged piperidine rings face each other across the intervening GC step with the charged centers separated by ∼15 Å. The width of the minor groove in A$_n$ sequences tends to decrease from their 5′-end, and this is observed in this complex, with the bulky piperazine ring of the drug located in the wider part of the groove close to the TpG step. Thus the orientation of the ligands in this structure appears to be, at least in part, sterically driven. Molecular dynamics (MD) simulations on the NMR refined structure, using an explicit solvent model, indicate that the intervening groove is occupied by solvent and that water molecules may be involved in mediating electrostatic interactions with the floor of the groove, as well as screening the two positive charges from each other.

In an attempt to shed some light on the origins of cooperativity in this system, we have used extended molecular dynamics simulations to study the free DNA, the 2:1 complex, and also the theoretical 1:1 complex. From the simulation data we have been able to calculate theromodynamic quantities relating to the two binding events and conclude that, in this case, cooperativity is entropy driven. It is well-established that the driving force for individual drug−DNA recognition events, especially by minor-groove binding ligands, can lie in entropic factors;[21] however, we now demonstrate that cooperative recognition may also have entropic origins. This is, to our knowledge, the first theoretical study of cooperativity in such a process, and the results illustrate the power of the latest generation of molecular simulation and analysis methods to offer explanations for perplexing experimental observations that are difficult to obtain by other means.

Methods

All simulations were performed with the AMBER 5.1 and AMBER 6 suites of programs. The AMBER-94/TIP3P force-field[22] with modifications[23] was used to describe the DNA and solvent. The HF/6-31G(d)/RESP methodology[24] was used to derive charges for Hoechst 33258. Missing force field parameters for the drug were adapted from comparable standard parameters. Starting structures for the free DNA and 2:1 complexes were taken from NMR data.[20] The systems were electrically neutralized by addition of sodium counterions and immersed in a periodic box of around 1760 water molecules (initial dimensions approximately 40 × 40 × 60 Å), optimized, thermalized, and equilibrated by using our standard multistage protocol.[25] The final equilibrated structures were then used to initiate three 5 ns unrestrained MD simulations at constant pressure (P = 1 atm) and temperature (T = 298 K). Shake was used to constrain all bonds, permitting a 2 fs time step for integration of Newton's equations. Energy analysis was done by using the MD implementation of the GB/SA method developed by Case and co-workers,[26] based on the MD trajectories obtained by using explicit solvent molecules. Finally, configurational entropies were computed by diagonalization of the Cartesian coordinate covariance matrix following the method described by Schlitter[27] and extensively tested in protein systems by Schafer et al.[28,29] Due to sampling issues,

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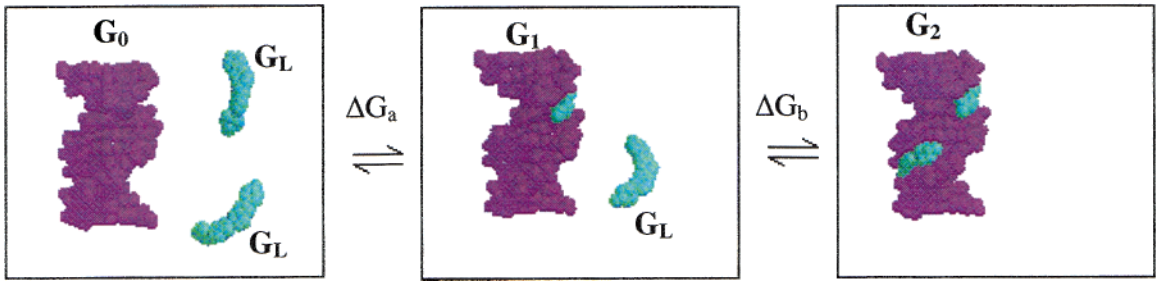
Scheme 2. Partitioning of the Free Energy Terms[a]



[a] $\Delta G_a = (G_1 + G_L) - (G_0 + 2G_L)$; $\Delta G_b = G_2 - (G_1 + G_L)$; $\Delta\Delta G = \Delta G_b - \Delta G_a = G_0 + G_2 - 2G_1$. G_0 is the free energy of the free DNA; G_L is the free energy of the free ligand; G_1. is the free energy of the 1:1 drug:DNA complex; and G_2 is the free energy of the 2:1 drug:DNA complex

the calculated entropies (S) are dependent on the length (t) of the trajectory that is analyzed, but tend clearly to a limit (S_∞) as the window width is increased. We find that entropies calculated for a range of window widths between 0.5 and 5 ns may be fitted well by using the empirical relationship:

$$S(t) = S_\infty - \frac{\alpha}{t^{2/3}} \quad (1)$$

Molecular Interaction Potential (MIP) calculations were performed on the time-averaged structures obtained from the equilibrated portions of each trajectory by using the methods previously described.[30] Both H33258 and a water molecule were used as probes. Hydration density maps were produced by integrating over the equilibrated portions of each trajectory using the methods previously described.[25,31]

Results

(a) General Characterization of the MD Simulations. All three simulations relaxed quickly from their initial conformations and remained stable over the 5 ns simulation periods, judging from root-mean-square deviation plots (not shown). Time-averaged structures were generated from the final 4 ns of each simulation. Those for the free DNA and 2:1 complex were found to be in excellent agreement with the NMR-derived structures. Heavy-atom root-mean-square deviations between the modeled and experimentally derived structures were 1.98 Å for the free DNA and 1.05 Å for the 2:1 complex. This was reduced to 1.65 and 1.03 Å respectively if terminal bases were excluded. Excellent agreement between experimental (NMR) and theoretically derived (MD simulation) helical parameters was also observed (Figure 2, cf. Figure 3 in ref 20). The MD simulations predict that the conformation of the DNA in the 1:1 complex does not differ greatly from that of the free DNA or the 2:1 complex. It shows the expected narrowing of the minor groove for the occupied A-tract, while the width of the unoccupied A-tract remains close to its free DNA value. This suggests that cooperativity does not relate to any dramatic conformational changes. The protocols used for these simulations have been in line with current practice; however, some workers have expressed concern that the relatively high DNA concentrations (approximately 0.1 M) and use of the Ewald method under periodic boundary conditions could lead to artificially restrained simulations. However, as reviewed by Cheatham and Kollman,[32] these worries appear to be unfounded.

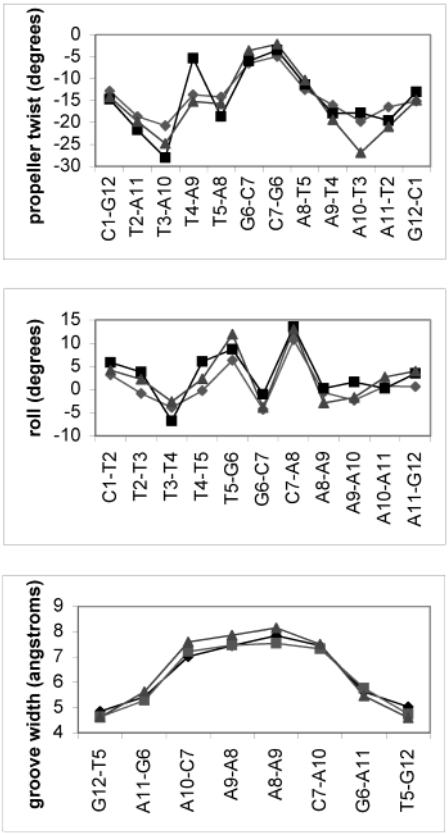


Figure 2. Propeller twist, roll, and minor groove widths (calculated using Curves[37]) for the time-averaged structures of the free DNA (diamonds), 1:1 complex (squares), and 2:1 complex (triangles).

(b) Thermodynamic Characterization of Cooperativity. The nature of the system and the equilibria involved allows us to calculate the difference between the free energy changes associated with the first and second binding events ($\Delta\Delta G$) directly from our simulations, i.e., without having to characterize the thermodynamics of unbound drug (Scheme 2).

It is useful to expand G, the total free energy of the system (including water and counterions) as shown in eq 2:

$$G = E^{\text{intra}} + G^{\text{solv}} - TS^{\text{intra}} \quad (2)$$

The first term, E^{intra}, is the internal energy of the solute (DNA or DNA−drug complex); the second term, G^{solv}, is the free energy of solvation of the solute; and the third term, S^{intra}, is the configurational entropy of the solute.

With use of Scheme 2 and eq 2, the free energy difference that constitutes cooperativity can be computed as shown in eq 3. Note that G^{solv} includes both enthalpic and entropic terms

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Table 1. Thermodynamic Parameters Calculated from the MD Simulations of the Free DNA (system 0), 1:1 Drug:DNA Complex (system 1), and 2:1 Drug:DNA Complex (system 2)[a]

system	$E^{int} + G^{solv}$	$\Delta(E^{int} + G^{solv})$	TS_∞ (system)	$T\Delta S_\infty$	TS_∞ (DNA only)	$T\Delta S_\infty$
free DNA	−4375.6 ± 0.2		830.6 ± 0.5		830.6 ± 0.5	
		29.0		−24.3		25.6
1:1 complex	−4404.6 ± 0.2		854.9 ± 0.3		805.0 ± 0.5	
		25.7		−34.8		13.1
2:1 complex	−4430.3 ± 0.2		889.7 ± 0.1		791.9 ± 0.5	

[a] All values are in kcal/mol ± standard errors, for $T = 300$ K

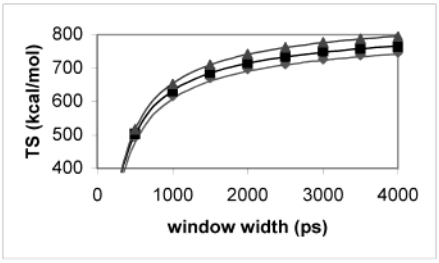

Figure 3. Effect of sample window width on the values of the configurational entropies, calculated by the method of Schlitter.[27] The symbol coding is as for Figure 2. The points are the experimental values, the lines are the results of the least-squares fit to the function given in eq 1.

related to the reorganization of the solvent (water and counterions).

$$\Delta\Delta G = (E^{intra} + G^{solv})_0 + (E^{intra} + G^{solv})_2 - (E^{intra} + G^{solv})_1 - T(S^{intra}{}_0 + S^{intra}{}_2 - 2S^{intra}{}_1) \quad (3)$$

By analyzing our trajectories using the GB/SA approach (see below) we can compute $(E^{intra} + G^{solv})$ terms, though we cannot unambiguously separate out the two contributions. In the following sections, we analyze the trajectories in terms of (i) internal energy and solvation components $(E^{intra} + G^{solv})$ and (ii) configurational entropy components (S^{intra}). Our purpose is to elucidate which one, or more, of these terms is responsible for the cooperativity observed in this system.

(i) Internal Energies and Solvation Terms. The internal energies, with solvation correction, of the free DNA, the 1:1 complex, and the 2:1 complex in each snapshot from the equilibrated portions of the trajectories were calculated by using the GB/SA method implemented in AMBER 6. The validity of this approach has been tested by Tsui and Case.[26] The resulting estimates (Table 1) give $\Delta\Delta(E^{intra} + G^{solv}) = 3.3 \pm 0.4$ kcal/mol. Thus, on the basis of enthalpy considerations alone (including a solvation correction), the interaction of H33258 with this DNA sequence is predicted to be somewhat anticooperative.

(ii) Calculation of Configurational Entropies and Free Energy Differences. Configurational entropies of the free DNA, the 1:1 complex, and the 2:1 complex were calculated from the dynamics data via Principal Component Analysis, using the method of Schlitter.[27] Entropy values obtained in this way are sensitive to the simulation length, and we therefore calculated S for various sample window widths and estimated S_∞ by fitting to eq 1 (see Figure 3 and Experimental Section). From the resulting values (see Table 1) we calculate $T\Delta\Delta S$ at 300 K to be 10.4 ± 0.7 kcal/mol. This implies that the binding of the first drug molecule to the DNA is associated with a considerably greater entropic penalty than the binding of the second. These calculations assume that changes in translational and rotational entropy can be ignored. This would appear reasonable. By statistical mechanics, the absolute translational entropy of a molecule is dependent on its mass, and its rotational entropy is dependent on the moments of inertia. Ligand binding will have a very small effect on these quantities. Indeed, quartz crystal microbalance experiments on related drug−DNA systems[33] indicate that ligand binding is associated with no change in the effective mass of the molecule, because an equivalent mass of tightly bound water is displaced from the minor groove in the process.

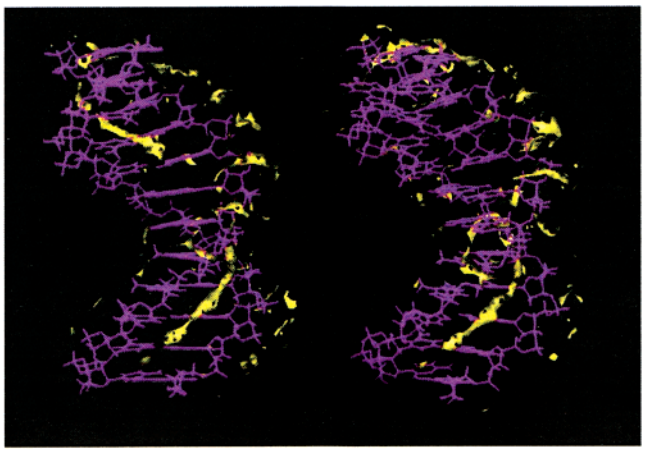

Figure 4. MIP plots for the time averaged structures of the free DNA (left) and 1:1 complex (right) obtained using H33258 as the probe. The map is contoured at a favorable interaction potential of −32 kcal/mol.

Combined with the value of $\Delta\Delta(E^{intra} + G^{solv})$ previously obtained, we therefore calculate $\Delta\Delta G$ for this system to be −7.1 ± 0.8 kcal/mol. The conclusions are clear: the result is in good agreement with the NMR titration estimates and we predict that, in this case, cooperativity is the result of the balance of entropic factors, which over-ride the small intrinsically anticooperative nature of the enthalpic terms involved.

(c) Qualitative Dissection of $(E^{intra} + G^{solv})$. The GB/SA approach does not allow the energetics of the system to be reliably decomposed into individual intramolecular energy and solvation terms. However, we have obtained some qualitative insight into these through examination of molecular interaction potential (MIP) maps[30] and hydration density maps.[25,31] Figure 4 shows the MIP map obtained for the time-averaged structures of the free DNA and the 1:1 complex, when a molecule of H33258 is used as the probe. For the free DNA, we see clear areas of density in both A-tracts. The density obtained for the unoccupied site in the 1:1 complex is very similar—there is thus no obvious change in the affinity of the second site for H33258 once the first molecule of drug is bound in the other site. Figure 5 shows the MIP maps obtained for the same structures when a water molecule is used as the probe. Clear "spines of hydration" are predicted in the unoccupied A-tracts of both structures, and again there is no evident difference between the

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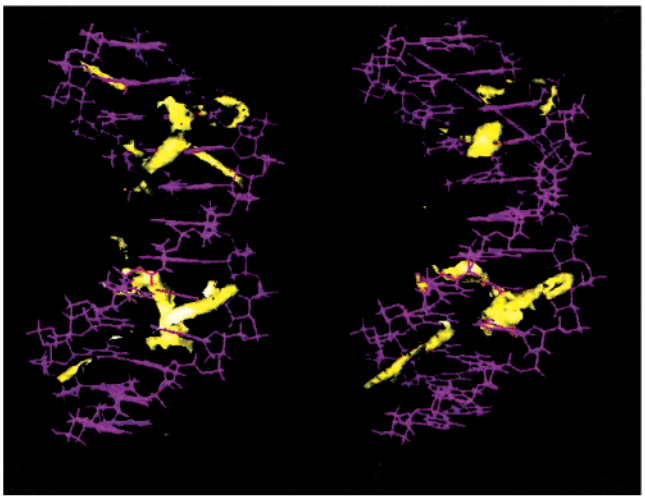

Figure 5. MIP plots for the time averaged structures of the free DNA (left) and 1:1 complex (right) obtained using a water molecule as the probe. The map is contoured at a favorable interaction potential of −10 kcal/mol.

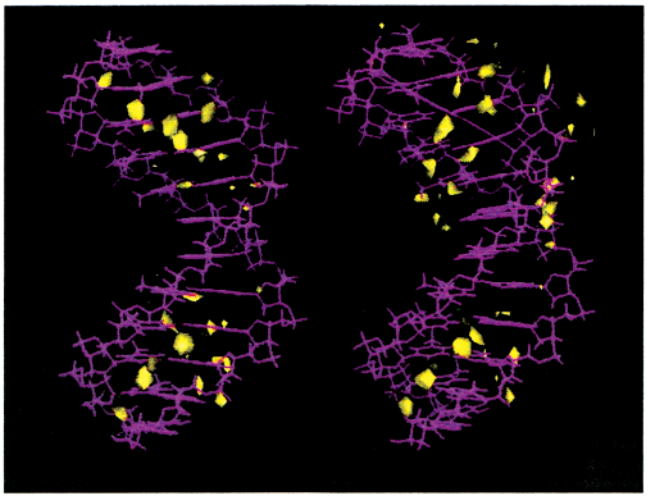

Figure 6. Hydration density maps for the free DNA and 1:1 complex, calculated by integrating water occupancies over the full 4 ns of the equilibrated trajectories. The map is contoured at a level corresponding to twice the density of the bulk solvent.

free DNA and 1:1 complex. This argues against cooperativity being the result of easier displacement of water from the second binding site, once the first molecule of drug is bound.

The MIP maps only relate to the enthalpic components of probe-target recognition, but hydration density maps reflect, qualitatively, free energies of solvation. The hydration density maps for the free DNA and 1:1 complexes are shown in Figure 6. Again, it is clear that, in areas not masked by the presence of a molecule of the drug, the hydration patterns for the DNA in the two situations are very similar.

These qualitative examinations suggest that the overall modestly positive value of $\Delta\Delta(E^{\text{intra}} + G^{\text{solv}})$ is not the result of a near canceling out of individual $\Delta\Delta E^{\text{intra}}$ and $\Delta\Delta G^{\text{solv}}$ terms that are large in magnitude but opposite in sign. This provides further support for our contention that we can regard the configurational entropy term as being the critical one.

(d)Further Dissection of the Entropic Term. To gain further insight into this result, we calculated the configurational entropy of the DNA component alone in the 1:1 and 2:1 complexes and compared these values with that for the free DNA (Table 1). For the binding of the first drug, we find $T\Delta S$ to be −25.5 ± 0.8 kcal/mol, while for the binding of the second drug it is calculated to be −13.1 ± 1.0 kcal/mol. For the DNA alone then, $T\Delta\Delta S$ is estimated to be 12.4 ± 1.1 kcal/mol. This is somewhat larger than that previously calculated for the whole drug−DNA system (10.4 ± 0.7), and suggests therefore that when the second drug binds, though the DNA itself is not greatly further restrained, the previously bound drug molecule loses some of its residual flexibility. To investigate this further, we initially examined the trajectories to see if the longitudinal motion of the drug in the A-tract minor groove was reduced by the introduction of the new positive charge further along this groove. However, analysis of selected drug−DNA distances sensitive to such motion revealed no significant difference between the 1:1 and 2:1 complexes. However, a general reduction in the flexibility of the drugs was evident from the calculation of simple coordinate root-mean-square fluctuations. In the 1:1 complex, the average root-mean-square fluctuation of the drug atoms is 0.28 Å, while for the 2:1 complex it is 0.23 Å (averaged over both drug molecules).

Table 2. Configurational Entropies for Top and Bottom Halves of the DNA in the Free, 1:1 and 2:1 Drug:DNA Complexes[a]

	top		bottom	
system	TS	$T\Delta S$	TS	$T\Delta S$
free DNA	366.3		368.4	
		−24.6		−4.6
1:1 complex	341.7		363.8	
		6.4		−16.9
2:1 complex	348.1		346.9	

[a] Values were calculated over the full 4 ns trajectories but not corrected to S_∞. All values are in kcal/mol, with T = 300 K.[36]

In any system of this type, where the binding sites for the two ligand molecules are physically separated, cooperativity relies on the ability of the receptor (in this case the DNA sequence) to pass information regarding the occupancy or otherwise of one site to the other. To examine this, we calculated the configurational entropies for each half of the DNA separately, in each of the free, 1:1, and 2:1 complexes. The results are shown in Table 2.

First we see that in the free DNA, the two halves of the DNA are calculated to have very similar entropies—a good test of the adequacy of the lengths of our simulations and the sampling. Binding of the first drug molecule is accompanied by a large reduction in the configurational entropy of that half of the DNA, as expected, but the "information" regarding occupancy is clearly also passed to the second, unoccupied, half of the DNA for we see that here also the configurational entropy of the DNA is noticeably reduced. We analyzed the contributions of each of the major principal components to the total entropy and find that this "information" transfer does not take place through major reductions in the flexibility of one or a few modes, but is the net result of small changes in the eigenvalues associated with many modes**.** Binding of the second drug molecule to this site results in further conformational restriction, but interestingly results in a "message" being passed back to the first occupied site that results in an increase in its configurational entropy. Again, we see that the calculated entropies for the two-half sites in the 2:1 complex are in close agreement, giving us some insight into the reliability of the approach. It is important to note that the entropy components calculated here cannot be summed to equate to the total values calculated previously, since the approach neglects the configurational entropy due to the relative motion of the two halves of the DNA.

(e) Mechanisms of Information Transfer. Analysis of the minor groove width variation associated with the principal

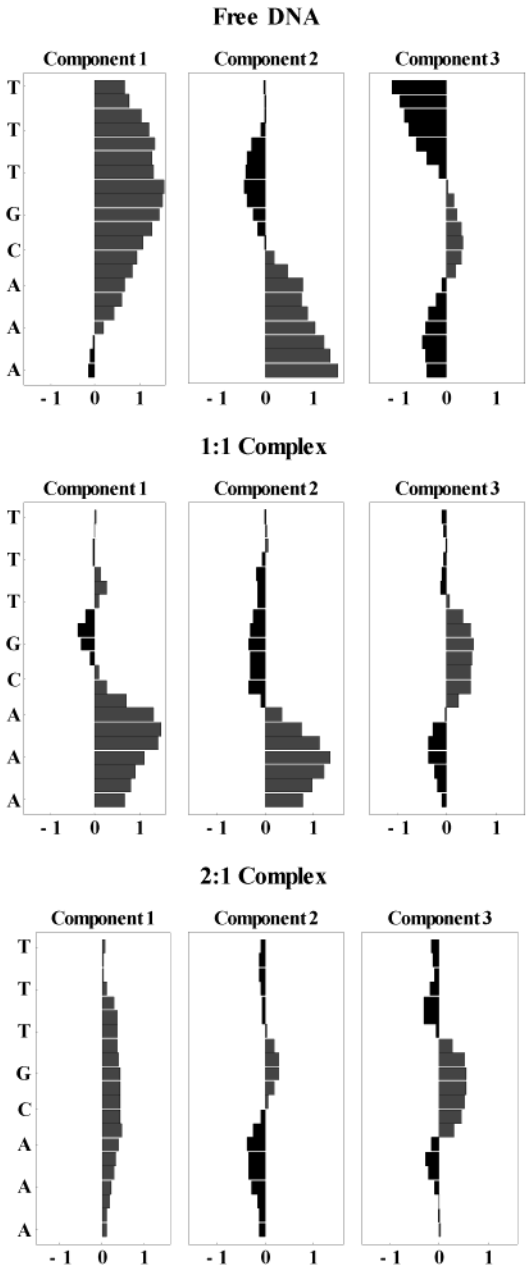

Figure 7. Patterns of groove width variation (in angstroms) associated with the top three eigenvectors of the dynamics of the free DNA and 1:1 and 2:1 complexes.

eigenvectors provides a striking picture of the underlying simplicity of DNA dynamics and possible mechanisms of information transfer between the sites. For the top three eigenvectors, structures of the free DNA were generated corresponding to the maximum and minimum values of the eigenvalues observed, as previously described.[34] Plotting the differences in groove width between these structures (Figure 7) reveals patterns reminiscent of the modes of vibration of a string. Clearly, interference with the motion of the groove at one binding site will be transferred through these modes to the other site. A similar analysis of the dynamics of the 1:1 and 2:1 complexes confirms this. In the 1:1 complex, the modes become highly asymmetric and higher harmonics predominate. In the 2:1 complex the symmetry is largely restored, and the lower harmonics are evident again. Although this remains to be further investigated, we hypothesize that the apparent compatibility of the 2:1 complex with the inherently symmetric modes of motion of the underlying DNA, compared to the incompatability of the asymmetric 1:1 complex, provides some qualitative explanation for the cooperative nature of recognition in this system.

Conclusions

The sequence-dependent interaction of DNA with molecules that bind in the minor groove involves a delicate interplay between enthalpic and entropic components of the recognition process. It has been shown through calorimetric studies[21] that in some cases the process is entropy-driven, being related to the solvation term. In general though, examples of cooperativity in DNA recognition appear to owe this characteristic to enthalpic factors, which are generally fairly evident, at least in qualitative form, from structure determinations. These typically reveal close physical contact between the two ligands, and/or a major structural deformation of the DNA that requires both ligands to stabilize it. In this case, NMR structure determination has shown that neither of these factors is operating. The MD studies reported here lead us to conclude that in this case, cooperativity is largely the result of the overall rigidity of the system. Binding of the first ligand restricts the flexibility of the DNA well beyond the actual binding site. Binding of the second ligand has little further effect. Both sites are already structurally fairly well predisposed toward ligand binding and the small adjustments required bear a modest enthalpic penalty and, though anti-cooperative, are outweighed by the entropic term.

The results of this investigation provide an example of the general hypothesis of allosteric communication without conformational change advanced by Cooper and Dryden.[35] These authors proposed that cooperativity could be the consequence of ligand-induced changes in the dynamic behavior of a receptor. Statistical thermodynamic arguments were used to calculate the possible magnitude of the cooperative effect that could be produced by purely dynamic changes. The approach was based on estimates of the increase in the frequency at each binding step, rather than measurements from computer simulation. The analysis showed that considerable differences in binding free energy could be obtained from changes in conformational flexibility alone, without the need for changes in the time-averaged structure of the macromolecule; others have presented similar arguments.[36]

The molecular origins of cooperativity in the absence of conformational change cannot be understood unless the dynamic properties of the system are taken into account. The results presented in this study illustrate the power of molecular simulation methods to investigate such phenomena, and highlight the general importance of flexibility in determining the properties of biomolecules such as DNA. Yet an element of rigidity, as well, is the key to the ability of this dodecamer to transmit "information" between the two drug binding sites. In ongoing investigations we are examining how this is modulated by the sequence and length of the intervening DNA.

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