Significance of Ligand Tails for Interaction with the Minor Groove of B-DNA

Bernd Wellenzohn, Wolfgang Flader, Rudolf H. Winger, Andreas Hallbrucker, Erwin Mayer, and Klaus R. Liedl
Institute of General, Inorganic, and Theoretical Chemistry, University of Innsbruck, A-6020 Innsbruck, Austria

ABSTRACT Minor groove binding ligands are of great interest due to their extraordinary importance as transcription controlling drugs. We performed three molecular dynamics simulations of the unbound $d(CGCGAATTCGCG)_2$ dodecamer and its complexes with Hoechst33258 and Netropsin. The structural behavior of the piperazine tail of Hoechst33258, which has already been shown to be a contributor in sequence-specific recognition, was analyzed. The simulations also reveal that the tails of the ligands are able to influence the width of the minor groove. The groove width is even sensitive for conformational transitions of these tails, indicating a high adaptability of the minor groove. Furthermore, the ligands also exert an influence on the B_I/B_{II} backbone conformational substate behavior. All together these results are important for the understanding of the binding process of sequence-specific ligands.

INTRODUCTION

Sequence-specific minor groove binding ligands (Ren and Chaires, 1999; Fishleigh et al., 2000; Geierstanger and Wemmer, 1995; Park and Breslauer, 1992; Goodsell et al., 1995; Sponar and Votavova, 1996; Wemmer and Dervan, 1997; Steinmetzer and Reinert, 1998) are able to influence the expression of specific genes (Gottesfeld et al., 1997; Dickinson et al., 1998; Wittung-Stafshede, 1998; Ho et al., 1994). Thus, small ligands such as Netropsin (Chen et al., 1996; Singh and Kollman, 1999; Zakrzewska et al., 1983; Zimmer et al., 1982; Duong and Zakrzewska, 1997; Perez and Portugal, 1990; Patel, 1982; Coll et al., 1989; Tarbernero et al., 1993; Kopka et al., 1985; Rentzeperis et al., 1995; Nunn et al., 1997; Lah and Vesnaver, 2000; Sriram et al., 1992b), Hoechst33258 (Sriram et al., 1992a; Spink et al., 1994; Squire et al., 2000; Teng et al., 1988; Carrondo et al., 1989; Vega et al., 1994), or small polyamides (Herman et al., 1999a,b; Kielkopf et al., 1998a) are of interest as antitumor, anti-viral, and anti-microbial agents. A great variety of such ligands have been synthesized and investigated with different experimental and theoretical methods. Now it is possible to distinguish between all four possible base pair steps (A-T, T-A, G-C, C-G) (Kielkopf et al., 1998b; Ellervik et al., 2000) in the minor groove. To recognize one specific DNA sequence out of the human genome (3×10^9 base pairs), the ligands have to interact at least with 17 base pairs (Thuong and Hélène, 1993). Thus, to improve the selectivity, the lengths of the ligand molecules are extended or hybrids of ligands are used (Ketterle et al., 1996; Perree-Fauvet and Gresh, 1994; Becker and Norden, 1999).

Netropsin and Hoechst 33258 have affinity for A+T-rich regions, and several studies of the drugs complexed with DNA are reported. The *N*-methyl piperazine (Pip), the two benzimidazole (Bz1 and Bz2), and the phenol (Phe) group are the four planar structural segments of the Hoechst 33258 ligand (Fig. 1). The torsion angles between these groups are named according to Quintana et al. (1991) $\zeta 1$, $\zeta 2$, and $\zeta 3$. Free rotation is possible around the connecting bonds. The Netropsin ligand consists of a guanidinium (Gua), two pyrrole (Py), and a propylamidinium (PrAm) part. Both molecules adopt a convex conformation (arc-like conformation) in DNA complexes, thus fitting exactly in the concave shape of the minor groove.

Footprinting experiments (Murray and Martin, 1988; Harshman and Dervan, 1985) performed on Hoechst33258 underline the A-T preference of the ligand. The exocyclic amino group of guanine prevents the binding of Hoechst-33258 in G+C-containing sequences although it has a tolerance for G-C base pairs at the end of the binding site. Recent molecular dynamics simulations of Netropsin (Wellenzohn et al., 2000b) and polyamide DNA (Wellenzohn et al., 2001) complexes and experimental investigations also proposed the importance of the ligand tails for recognition processes. Cheryl et al. (2000) suggest that the tails are responsible for the binding orientation of small ligands, and Becker and Norden (1999, 2000) attribute sequence specificity to the interaction of a cationic piperazinium tail with the minor groove (Ren et al., 1999; Wilson et al., 1985). Thus, an exact structural knowledge of the interaction behavior between the ligand tails and the DNA is of extraordinary interest in the design of new ligands.

Behind the direct readout arranged through ligand-DNA contacts the indirect readout also contributes to sequence specificity and selectivity (Neidle, 1997; Dickerson, 1998; Giese et al., 1997; Chen and Prohofsky, 1995; von Hippel, 1994; Strauss et al., 1996; Bareket-Samish et al., 1998; Wenz et al., 1996; Steitz, 1993; W. Flader, B. Wellenzohn, R. H. Winger, A. Hallbrucker, E. Mayer, and K. R. Liedl,

Received for publication 20 February 2001 and in final form 24 May 2001.
Address reprint requests to Dr. Klaus R. Liedl, Department of Theoretical Chemistry, Institute of General, Inorganic, and Theoretical Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria. Tel.: 43-512-507-5164; Fax: 43-512-507-5144; E-mail: klaus.leidl@uibk.ac.at.

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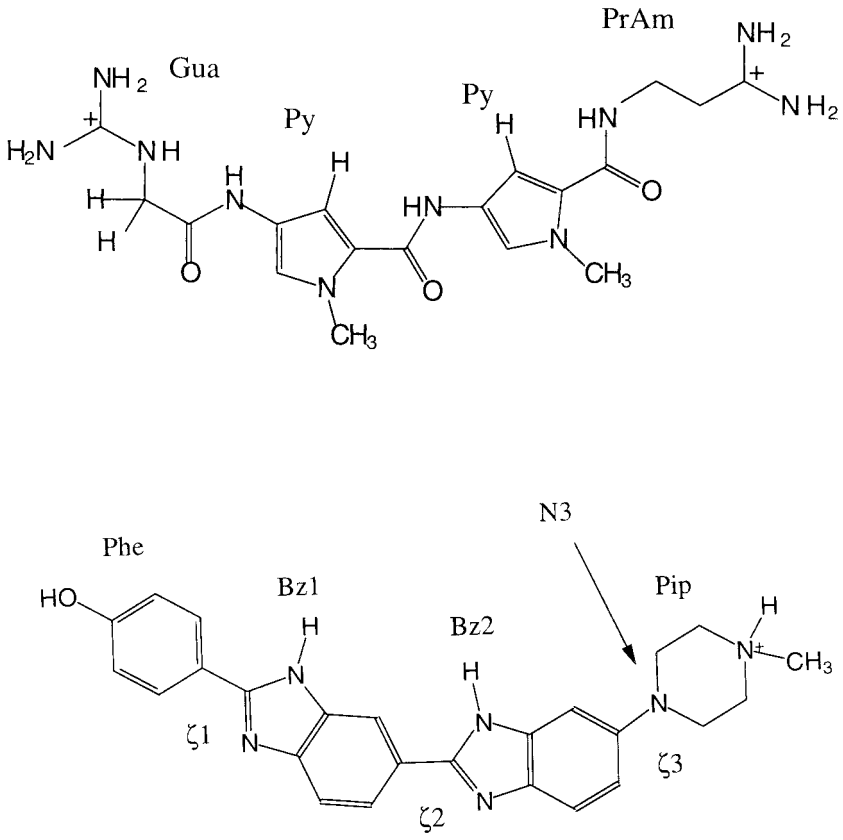

FIGURE 1 Chemical structures of the minor groove binders Netropsin (*top*) and Hoechst33258 (*bottom*). The abbreviations of the different moieties are explained in the text. Both molecules are drawn in their convex (arc-like) conformation, which they adopt in DNA complexes, exactly fitting in the concave shape of the minor groove. The torsion angles between the Hoechst33258 moieties are named according to Quintana et al. (1991) $\zeta 1$, $\zeta 2$, and $\zeta 3$. The arrow indicates that the atomic type N3 was used for this nitrogen (explained later in the text).

submitted; Flader et al., 1995; Gehring et al., 1994; Bewley et al., 1998). Indirect readout can be mediated by means of changes in structural parameters such as bending, unwinding, and the groove width. Two different models are used to explain the heterogeneity in the minor groove. One model explains the groove width by the repulsion of the negative phosphate groups, thus proposing an influence of positive charges on the minor groove width (Hamelberg et al., 2000; Shui et al., 1998; Tereshko et al., 1999; Feig and Petitt, 1999; Hud and Feigon, 1997; Young and Beveridge, 1998). The second model makes the short-range interaction of DNA bases responsible for the size of the minor groove (Wing et al., 1980; Drew and Dickerson, 1981; Chiu et al., 1999).

The B_I/B_{II} conformational substates are defined by the ϵ and ζ angles of the B-DNA backbone or by the angle difference ($\epsilon - \zeta$). In the B_I state the corresponding ϵ and ζ angles are between 120° and 210° (*trans*) and 235°-295° (*gauche*$^-$), respectively; for B_{II}, the ϵ angle lies between 210° and 300° (*gauche*$^-$), ζ between 150° and 210° (*trans*) (Schneider et al., 1997; Berman, 1997; Hartmann and Lavery, 1996). The angle difference ($\epsilon - \zeta$) is close to −90° for B_I and +90° for B_{II} phosphates (Fratini et al., 1982). Molecular dynamics simulations compared with experimental results have shown that force fields are able to describe the B_I/B_{II} substate pattern in a correct way (Winger et al., 1998; Rüdisser et al., 1997; Pichler et al., 1999, 2000a,b). The complexation of the minor groove with ligands influences the B_I/B_{II} behavior of the DNA. It has been proposed that these conformational substates are able to contribute to sequence recognition (van Dam and Levitt, 2000; Song et al., 1997; Wellenzohn et al., 2000b, B. Wellenzohn, W. Flader, R. H. Winger, A. Hallbrucker, E. Mayer, and K. R. Liedl, submitted; W. Flader, B. Wellenzohn, R. H. Winger, A. Hallbrucker, E. Mayer, and K. R. Liedl, submitted; Pichler et al., 2000a).

We performed two 5-ns molecular dynamics simulations of complexes of the Drew Dickerson dodecamer (d(CGCGAATTCGCG)$_2$). In the first simulation the dodecamer is complexed with Netropsin and in the second simulation with Hoechst33258. As reference we use a 10-ns simulation of the unbound dodecamer. The comparison of all three simulations allows us to investigate on the one hand the common effects induced by minor groove binding ligands

on DNA, and on the other hand we are able to detect differences between Hoechst33258 and Netropsin. Both Hoechst33258 and Netropsin undergo structural transitions at the end of the molecule. These structural changes of the tails of the ligand are not separable from those of the DNA because they induce changes in the minor groove width. Thus, the results indicate that the minor groove width exhibits great flexibility and changes the structure to fit the ligand exactly in the groove. In such a case a rigid ligand should lead to an entropic penalty due to stiffening of the DNA by complexation, or if the DNA keeps its pliability the direct interaction is weakened. This structural and dynamic knowledge of the complexation is of importance in the ligand design because, for example, the interaction of a piperazine part with the minor groove is able to introduce sequence specificity to intercalating ligands (Becker and Norden, 1999). The two minor groove binders are also able to change the pattern of the B_I/B_{II} substates, supporting recent suggestions of the influence of these substates in sequence recognition (van Dam and Levitt, 2000; Song et al., 1997; Wellenzohn et al., 2000b; W. Flader, B. Wellenzohn, R. H. Winger, A. Hallbrucker, E. Mayer, and K. R. Liedl, submitted; Pichler et al., 2000a,b).

METHODS

Molecular dynamics simulations of DNA and DNA complexes are able to provide complementary information to experimental evidence. Thus, molecular dynamics simulations are an essential tool in the field of biomolecular research. The inclusion of the long-range interactions via the Ewald summation in the form of the particle mesh Ewald method leads to stable B-form DNA trajectories. We performed two simulations of DNA complexes (simulations A and B) and one reference simulation of the unbound DNA (simulation C) (Fig. 2).

Simulation A

As a starting point the crystal structure of the Nt/d(CGCGAATTCGCG)$_2$ complex was used (Nt represents Netropsin). The structure has the protein data bank (PDB) code 1D86. Each strand of the DNA has 11 PO_4^- anions. The Netropsin molecule has two positive charges. To achieve electroneutrality 20 Na^+ counterions were added using the program CION of the AMBER (Case et al., 1997) package. Subsequently, solvation of the DNA with TIP3P Monte Carlo water boxes requiring a 12-Å solvent shell in all directions resulted in a system with the dimension $67.1 \times 50.6 \times 48.7$ Å^3 containing 4642 water molecules. The corresponding Γ-value (water/nucleotide) is 193.4. The simulation was carried out using the AMBER5 (Case et al., 1995) package with the all-atom force field of Cheatham et al. (1999). The procedure of the parameter development for the ligand has already been described (Wellenzohn et al., 2000b). Standard protocols (Young et al., 1997a,b; de Souza and Ornstein, 1997a,b; Winger et al., 1998) were adapted for our needs. At the beginning, minimizations were carried out with harmonic restraints on DNA and counterion positions. The restraints were stepwise relaxed, and at the end, a 500-step minimization without restraints was performed. For equilibration the system was heated from 50 K to 300 K during 10 ps under constant volume conditions and harmonic restraints. Subsequently, the restraints were once again relaxed, and finally an unrestrained 5-ps equilibration was carried out. After this procedure the system was switched to constant temperature and pressure and simulated for 5 ns.

Simulation B

The procedure described for simulation A was also used for simulation B. As a starting point for the Hoe/d(CGCGAATTCGCG)$_2$ complex (Hoe represents Hoechst33258) the x-ray structure with the NDB-code gdl012 (Quintana et al., 1991) was used.

Simulation C

For the simulation of the unbound DNA (used as reference simulation) a similar protocol as described for simulations A and B was used that is described elsewhere (W. Flader, B. Wellenzohn, R. H. Winger, A. Hallbrucker, E. Mayer, and K. R. Liedl, submitted). All simulations produced the B-form of DNA. This is consistent with recent infrared spectroscopic studies of the Drew Dickerson dodecamer that have shown that it persists in the B-form even at low water activity (Pichler et al., 2000a,b).

RESULTS

The energy of the systems was stable during the simulations and the root mean square values with respect to the starting structures were in the range of ~2–3 Å and showed no drift. An analysis of the ligands during the simulation indicates that the tails of the ligands undergo structural transitions. Structural transitions found in the Netropsin complex were recently published (Wellenzohn et al., 2000b), so we concentrate on the changes in the Hoechst33258 ligand. The torsion angles $\zeta 1$, $\zeta 2$, and $\zeta 3$ of the Hoechst33258 molecule are shown in Fig. 3, pointing out transitions only in $\zeta 3$. Thus, the piperazine ring rotates while the rest of the molecule stays in the starting x-ray (Quintana et al., 1991) conformation during the simulation.

The piperazine end of the molecule extends partially into the GC region of the DNA. The minor groove of this GC region is wider than that in the A-tract, which may be the explanation of the enhanced flexibility of this side of the Hoechst33258 ligand. Fig. 3 indicates that three distinct substates occur, and representative snapshots are shown in Fig. 4. The detailed analysis of such snapshots leads to the conclusion that a nitrogen inversion is the origin of the structural transitions. The nitrogen transition is responsible for only two substates, but as seen above three different substates occur. The third substate arises from an additional torsion that occurs only in one of the two nitrogen inversion states.

The atomic type for the inverting nitrogen was chosen to be N3 (see Figs. 1 and 4), which represents an sp^3-hybridized nitrogen (MD et al., 1995; Cheatham et al., 1999). This is in contrast to the types normally used for a nitrogen bound to an aromatic system (sp^2-hybridization) taking into account ab initio calculations (Sponar et al., 1996). The calculations of Sponar suggest the nonplanarity of amino groups that are bound to aromatic systems such as in aniline or in the nucleic acid bases. The nonbonded interaction energy between the piperazine and the DNA does not alter significantly during the structural changes, suggesting entropy as the driving force for the transitions.

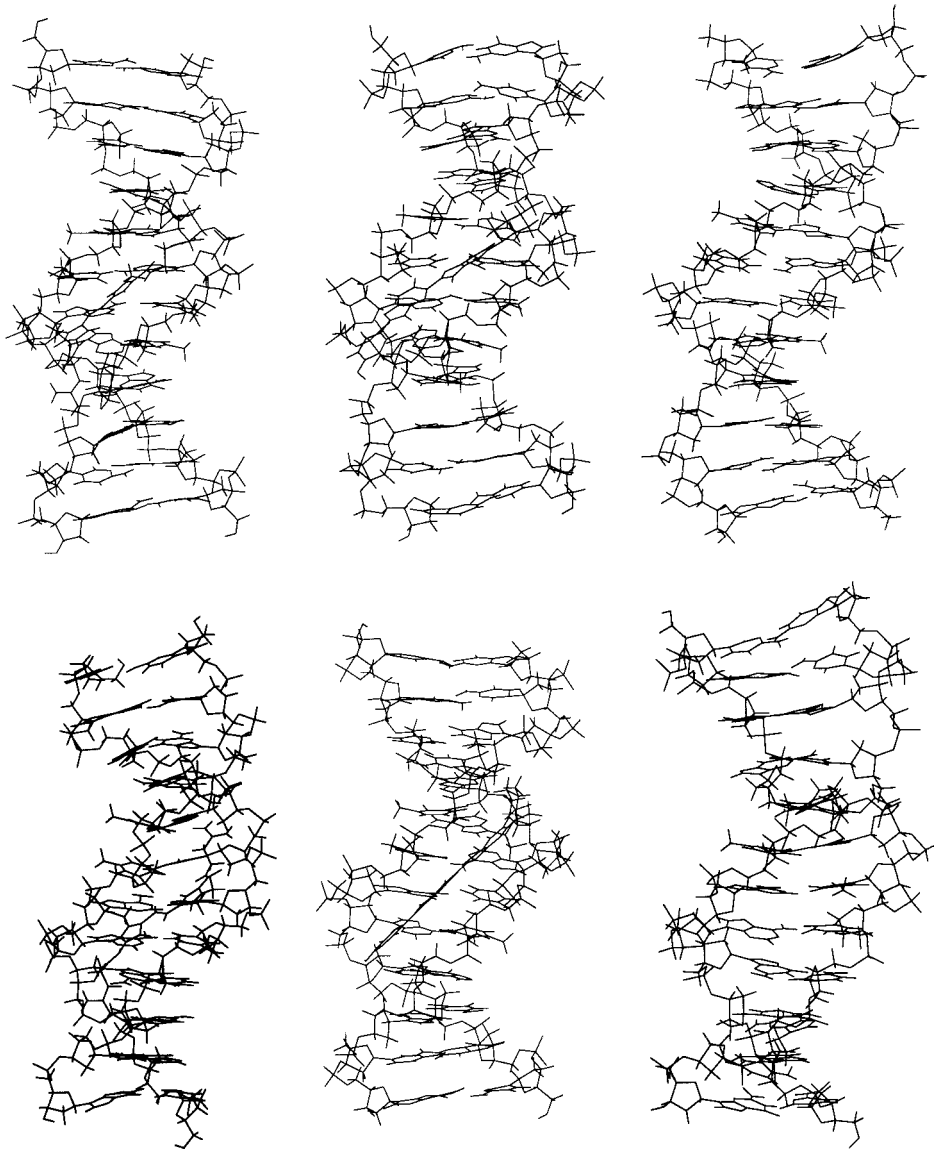

FIGURE 2 The top graphs show the starting points of the simulation. On the left side the Drew Dickerson dodecamer is complexed with Netropsin (simulation A), in the middle the minor groove bound ligand is Hoechst33258 (simulation B), and on the right side the dodecamer is unbound (simulation C). The structures on the bottom show the respective snapshots of the simulations at 5 ns.

An analysis of the Hoechst33258 ligands in the x-ray structures of DNA ligand complexes indicates that such nitrogen inversions as above described are also experimentally observed. Fig. 5 shows the piperazinium part of three different experimental structures taken from the PDB, and at both piperazine nitrogens inversions occur. The nitrogen inversions torsions about $\zeta 3$ are also found in the different experimental structures. Thus, our simulations indicate that the piperazinium tail of Hoechst33258 must be considered as a highly flexible part, explaining the structural variability in the crystallographic structures.

The contribution to sequence specificity of such piperazine tail interactions with the minor groove of DNA have been reported recently (Becker and Norden, 1999, 2000; Ren et al., 1999), which underlines the importance of this exact structural and dynamical understanding.

The consequence of minor groove binding on the groove width is shown in Fig. 6, and it indicates that the two ligands affect the minor groove in different ways. In the case of Netropsin the complexation leads to a reduction of the minor groove width with the exception of the distances P21-P8 (number 4) and P20-P9 (number 5). In this region Netropsin is bound to the DNA with the two pyrrole parts preventing a too small minor groove by steric hindrance. The minor groove widening of this part of the DNA with respect to the unbound case is not significant, indicating that

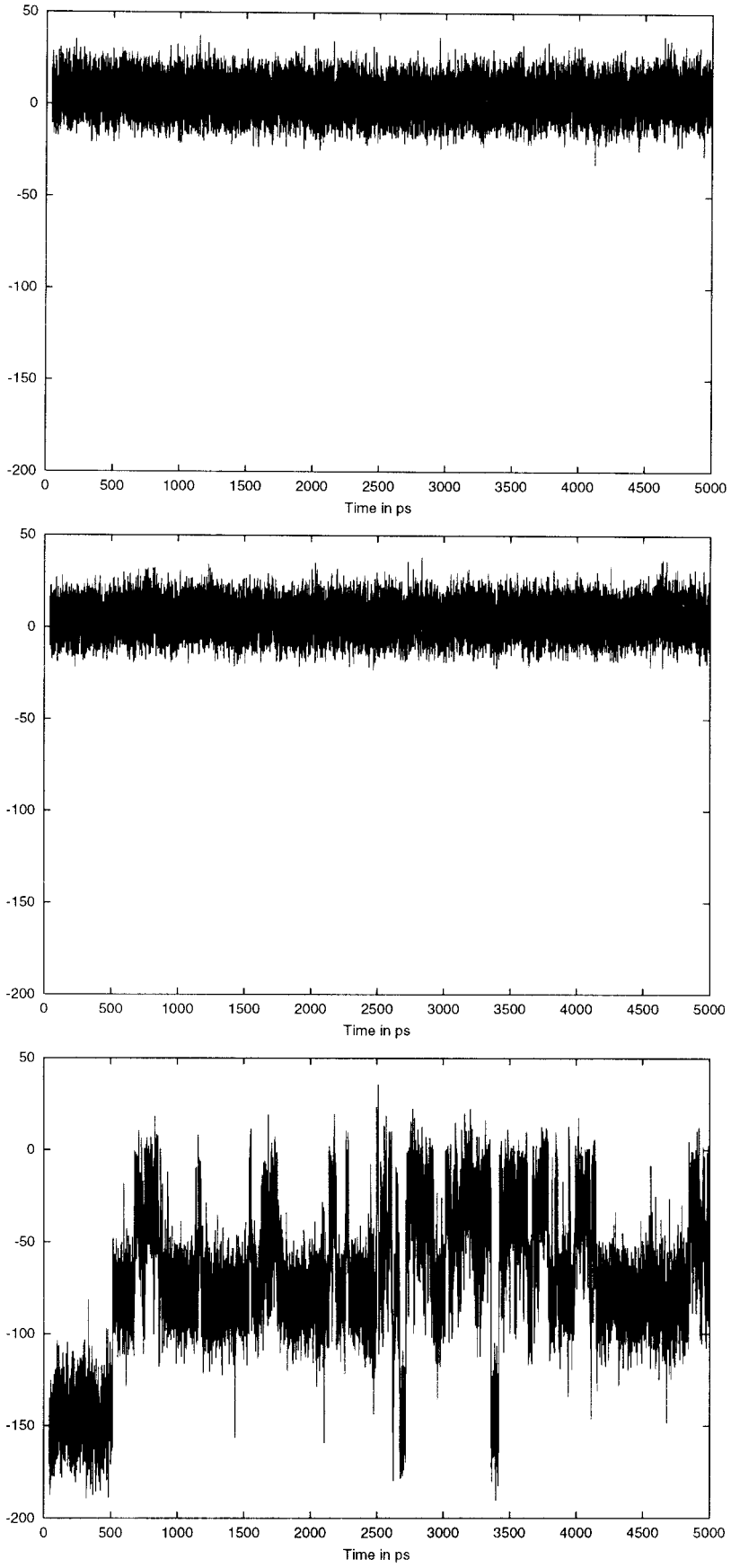

FIGURE 3 The torsion angles $\zeta 1$ (*top*), $\zeta 2$ (*middle*), and $\zeta 3$ (*bottom*) of Hoechst 33258 during the simulation. $\zeta 1$ and $\zeta 2$ stay stable during the whole simulation, indicating that the molecule stays in the planar starting conformation, and $\zeta 3$ exhibits structural transitions.

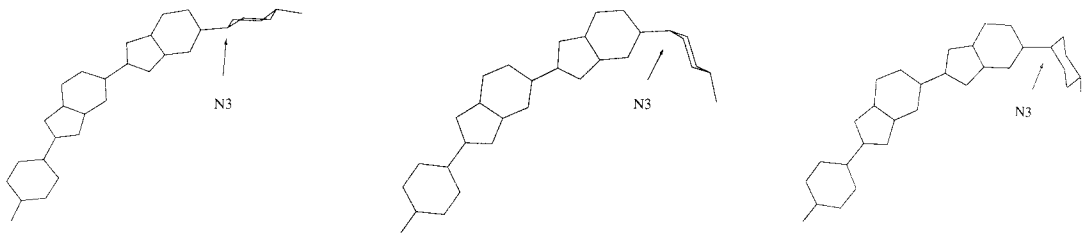

FIGURE 4 Three representative snapshots indicating the structural transitions at the piperazine tail of the molecule. The arrows mark the position at which the nitrogen inversion takes place. The structure on the left is the starting structure and represents the lowest substate in Fig. 3, the structure in the middle represents the middle substate, and the right structure represents the highest substate of Fig. 4. The difference between the left and the middle or right structure is due to the nitrogen inversion. The middle and the right structures are in the same nitrogen state but differ in a torsion between the piperazine moiety against the benzimidazole.

FIGURE 5 The graph shows the piperazinium part of three different x-ray structures. The left structure is the structure with the PDB code 1D44, the structure in the middle has 127D, and the PDB code of the right structure is 128D. The two piperazine nitrogens are in gray, and the R indicates the rest of the Hoechst33258 molecule. A nitrogen inversion of the right piperazine nitrogen converts the left structure to the middle structure, and a further nitrogen inversion of the left (not as distinctive as the first one) nitrogen converts the middle to the right structure.

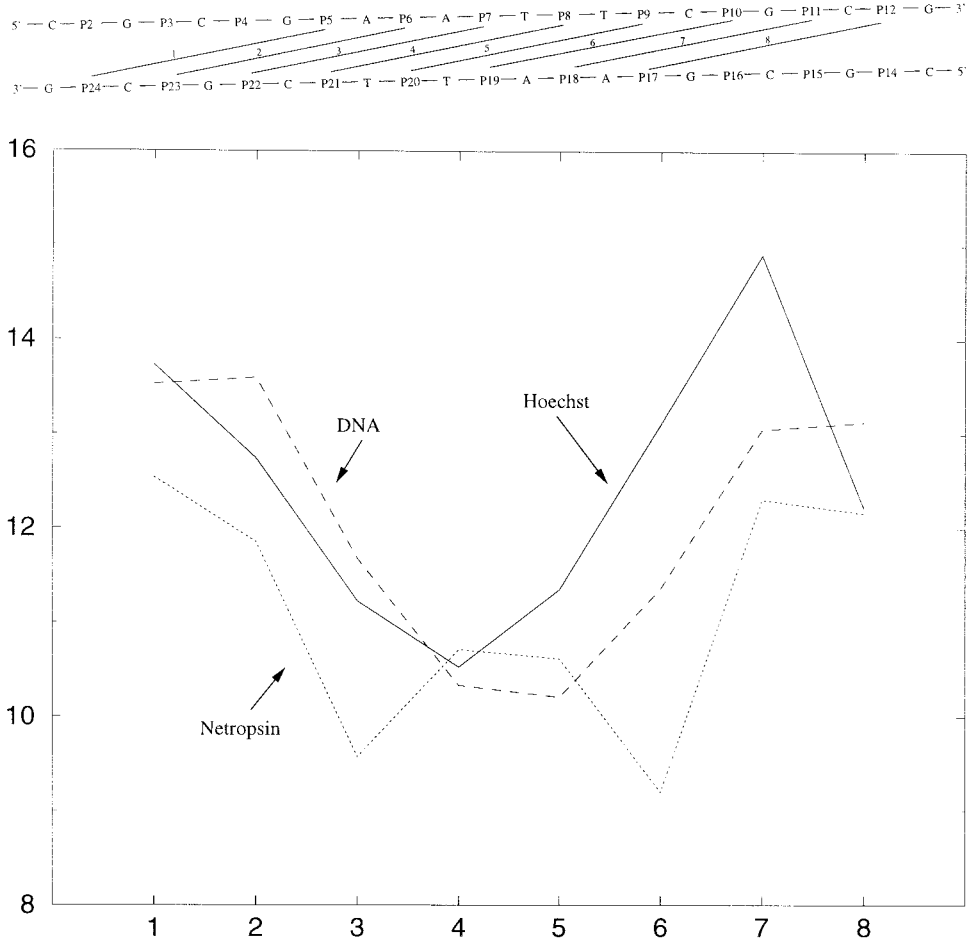

FIGURE 6 The bottom plot shows the average values of the groove width (in Å) for the unbound Drew Dickerson dodecamer (labeled DNA), complexed with Netropsin and complexed with Hoechst33258. The schematic picture on the top defines the P-P distances used for calculating the groove width.

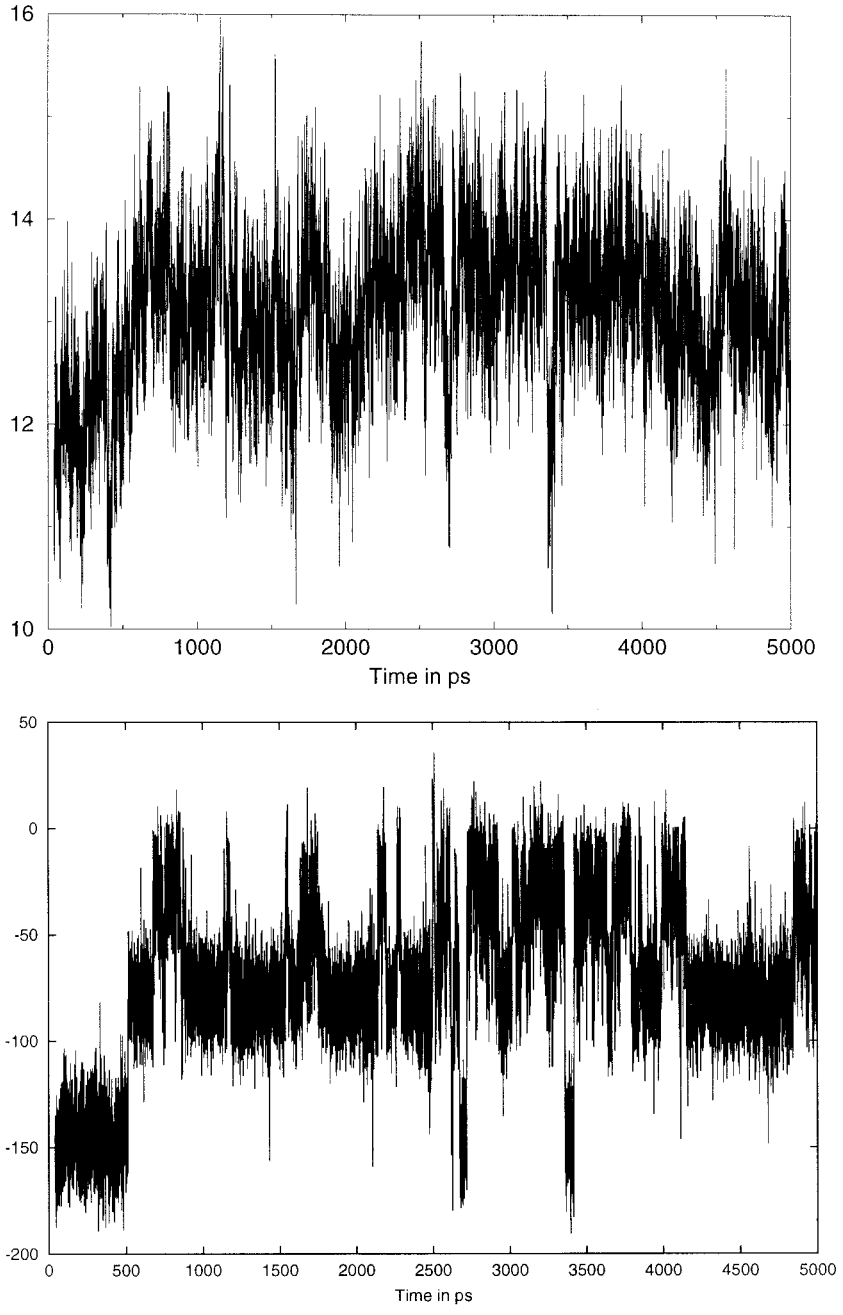

FIGURE 7 The top graphs show the distance between P19-P10 as indicator for the groove width, and the plot on the bottom shows $\zeta 3$. The correlation coefficient between both curves is 0.495, showing that the structural transitions are simultaneously performed. The mean value of the groove width of the first 500 ps is ~1.2 Å smaller than after the first 500 ps.

the pyrroles fit very well in the groove. In all other cases, complexation with Netropsin reduces the groove width. This effect is extended over the whole DNA and not restricted to the binding region, having also implications for additional binding sites.

The Hoechst33258 ligand exerts a different influence on the groove width. In contrast to the Netropsin case, the binding of the ligand induces a widening in the minor groove. This widening is introduced only on this end of the DNA on which the piperazine part is bound. As described above the piperazine undergoes structural changes during the simulation, and a comparison of these changes with the time dependence of the groove width shows (Fig. 7) that the structural changes also affect the groove width. The correlation coefficient calculated between the structural changes indicated by $\zeta 3$ and the

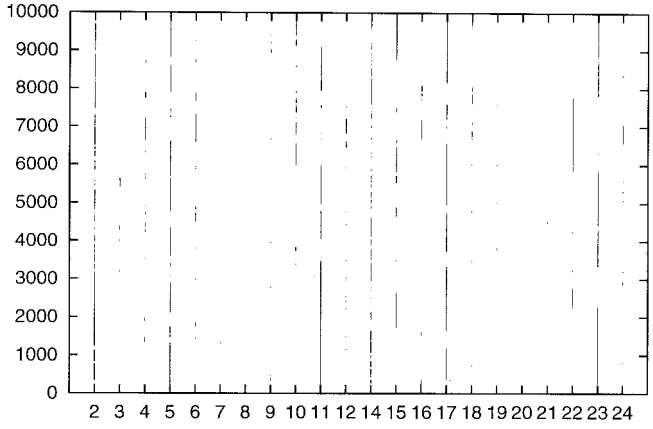

FIGURE 8 The B_I/B_{II} substate behavior of the unbound Drew Dickerson dodecamer as a function of time. The time (in ps) the respective ϵ-angle is in substate B_{II} is marked by a black line/dot. The enumeration of the phosphates is shown in Fig. 6 (*top*) and is according to Kopka et al. (1985).

groove width of P19-P10 (number 6) is ~0.5. The structural transitions after 500 ps lead to a widening of the minor groove of ~1.2 Å.

The correlation between the structural transitions in the ligand and the minor groove width could also be observed in the case of complexation with Netropsin (not shown). The interaction energy between the piperazine and the DNA does not alter significantly during the structural changes, suggesting entropy as the driving force for the transitions. Thus, a rigid tail of the ligand should lead to an entropic penalty due to stiffening of the DNA by complexation, or if the DNA keeps its pliability the direct interactions should be weakened.

The B_I/B_{II} conformational substate behavior of the three simulations also differs in several points, which underlines the ability of DNA to react on distortions such as binding of ligands. In the uncomplexed DNA (Fig. 8), the A-tract (numbers 6–9 and 18–21 in Figs. 8 and 9) in which the ligands bind exhibits almost no B_{II} in agreement with known results (Winger et al., 1998). The simulation also indicates that two successive base pairs are never in the B_{II} substate at the same time.

In contrast to the unbound state, in the case of the DNA-ligand complexes, the A-tracts contain phosphates in the B_{II} substate (Fig. 9). This is an unusual behavior of such base pair steps and therefore assigned to the binding of the ligand. Thus, we conclude that minor groove binding ligands are able to influence the B_I/B_{II} substate pattern. The differences in the B_I/B_{II} behavior between Netropsin and Hoechst33258 show that the ligands differ in the way they influence the backbone conformations. In a recent simulation (Wellenzohn et al., 2001) it was shown that the binding of two polyamides bound to the same minor groove position freeze out the DNA-backbone flexibility. In contrast to this, the binding of Netropsin and Hoechst33258 rather leads to

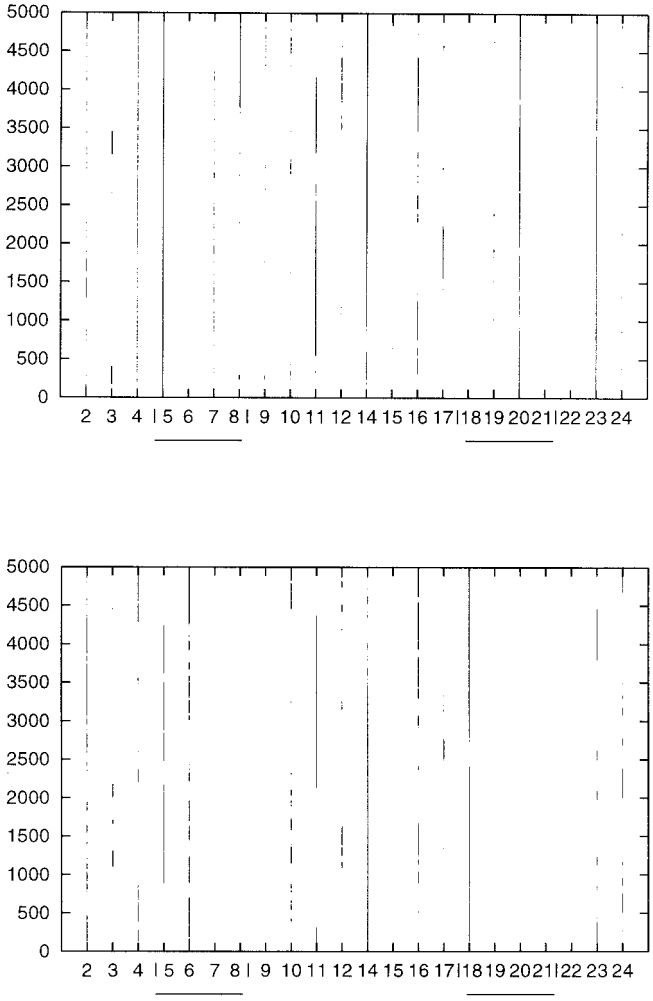

FIGURE 9 The B_I/B_{II} substate behavior of the $(CGCGAATTCGCG)_2$ dodecamer complexed with Netropsin (*top*) and bound with Hoechst33258 (*bottom*) as a function of time. The regions to which the ligands bind are indicated by the lines.

an enhanced B_I/B_{II} substate transition flexibility. The freezing-in of the phosphates was explained as a result of an optimization of the nonbonded contacts, which are the main contributor to the binding of minor groove ligands. Together with our new results we believe that the freezing-in in the polyamide-DNA complexes is due to steric hindrance. The two ligands Netropsin and Hoechst33258 are bound as monomers and are thus sterically much less demanding than complexation with two polyamides and therefore do not reduce the backbone conformational flexibility.

As described above in uncomplexed DNA no successive base pairs are at the same time in B_{II}. In the complexes this condition is fulfilled only with some exceptions. In the Netropsin simulation the successive phosphates 4 and 5 are in the B_{II} state over a long period (Fig. 9, *top*) in the simulation. A detailed analysis of both ϵ angles indicated

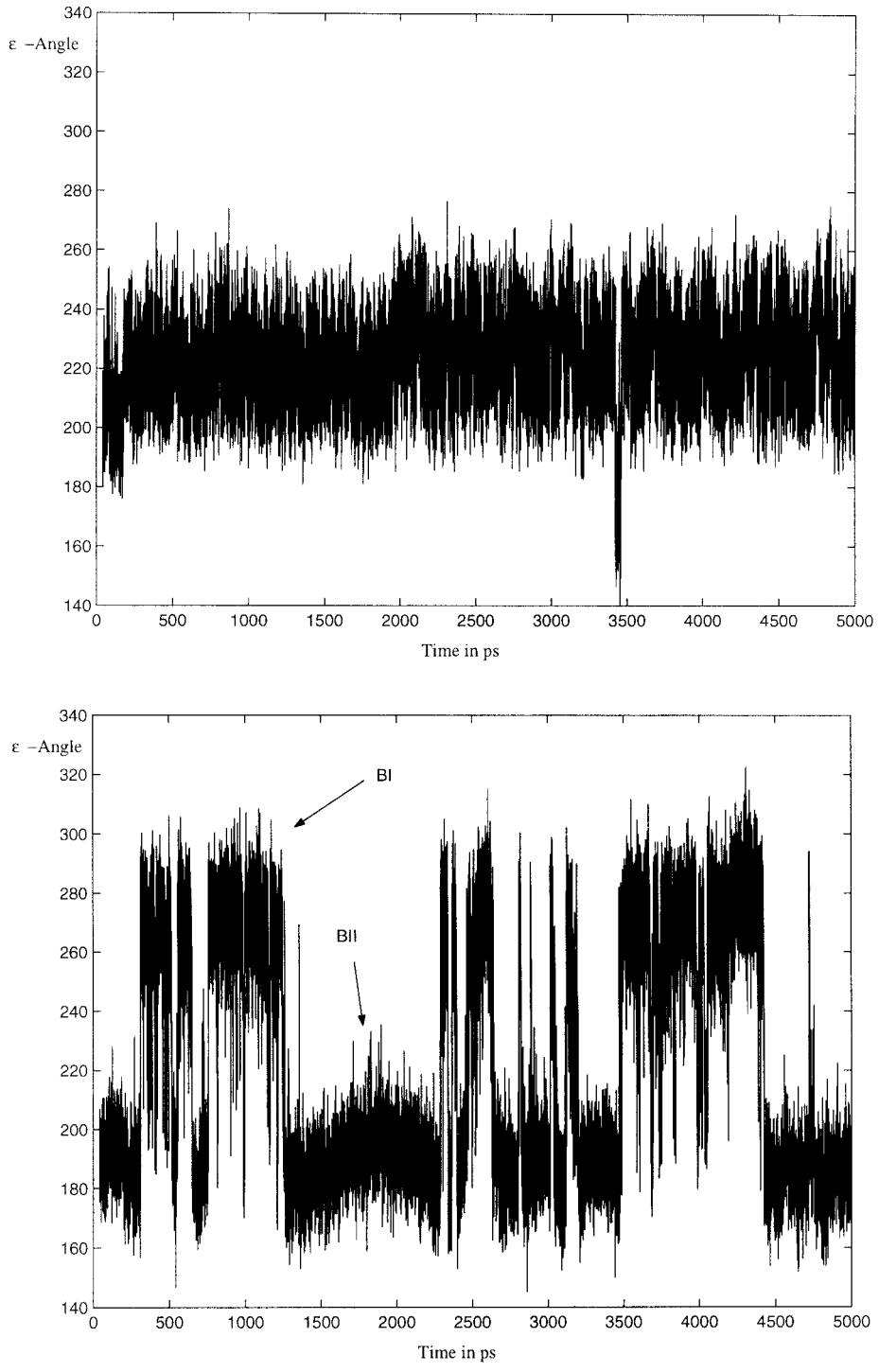

FIGURE 10 The top graph shows the ϵ-angle of P4 as a function of the time, and the bottom graph shows the respective angle of P16. The mean value of both angles is at ~220°, indicating that P4 is in between the B_I and B_{II} substate.

that P5 is in B_{II} during the whole simulation and that P4 is neither in a stable B_{II} nor in B_I. The mean value of this ϵ angle is at 220°, which is between the ϵ angles of the two substates (Fig. 10, *top*). For comparison the ϵ angle of P16 (shown in Fig. 10, *bottom*) is approximately half the time in B_I and half the time in B_{II}. Thus, its mean value is about the same (220°) as that of P4 (Fig. 10, *top*). Recently made x-ray studies also found DNA-phosphates that do not belong to either the B_I or B_{II} conformational group (Schuerman and Van Meervelt, 2000).

It is worth pointing out that the B_I/B_{II} substate pattern reported as a function of time in our first study of uncomplexed Drew Dickerson dodecamer over 3 ns shows about the same behavior as shown here in Fig. 8. The most pronounced differences being the B_{II} substate population at P11, although the force field was changed from the Cornell et al. (1995) force field to the improved one of Cheatham et al. (1999). The changes in the B_I/B_{II} substate behavior on complexation with Netropsin or Hoechst33258 shown in Figs. 8 and 9 for the same force field are much more pronounced. This is strong support that these changes are caused by interaction with the ligand and not by the force field applied in the simulations. All together, complexation influences these B_I and B_{II} backbone conformational substates, and thus these substates may be able to contribute to sequence-specific binding of a protein

SUMMARY AND CONCLUSION

We performed three simulations of the Drew Dickerson dodecamer alone and complexed with Hoechst33258 and Netropsin. The tails of the ligands undergo a variety of structural transitions during the simulation in agreement with the structural variability of these tails in the crystallographic studies. The conformational changes of the tails are correlated with the time dependence of the groove width. Thus, the results indicate that the minor groove exhibits a great flexibility, fitting the ligand exactly in the groove. A more rigid ligand tail would lead either to entropic cost due to stiffening of the DNA by complexation or, if the DNA keeps it pliability, weakening of the direct contacts. Furthermore, the binding of the ligand influences the B_I/B_{II} conformational substate behavior, having possible implications for protein recognition processes. All these results are of importance for the understanding of the binding process valuable in the design of new sequence-specific minor groove binding ligands.

This work was supported by a grant of the Austrian Science Fund (grant P13845-TPH).

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