A Polarized-Light Spectroscopy Study of Interactions of a Hairpin Polyamide with DNA

Christina E. B. Caesar,* Richard Johnsson,† Ulf Ellervik,† Keith R. Fox,‡ Per Lincoln,* and Bengt Nordén*
*Department of Chemistry and Bioscience, Chalmers University of Technology, S-41296 Gothenburg, Sweden;
†Department of Bioorganic Chemistry, Lund University, S-221 00 Lund, Sweden; and ‡Division of Biochemistry and Molecular Biology, School of Biological Sciences, University of Southampton, Southampton SO16 7PX, United Kingdom

ABSTRACT We here study the interactions of a polyamide with large DNA, and compare to those of minor groove binder distamycin (DST), including high ligand/DNA binding ratios. Specific as well as nonspecific binding is probed using polarized-light spectroscopy combined with singular value decomposition analysis. Circular and linear dichroism data confirm binding geometries consistent with minor groove binding for both of the ligands. Interestingly, at high and intermediate ligand/DNA ratios the polyamide exhibits no significant sequence discrimination between mixed-sequence (calf thymus) and AT DNA as compared to DST. Each ligand is concluded to exhibit two different binding modes depending upon ligand/DNA ratio and nucleo-base sequence. At high binding ratios, distinct differences between the ligands are observed: circular dichroism spectra exciton effects provide evidence of bimolecular interactions of the polyamide when bound to AT-DNA, whereas no effects are seen with DST or mixed-sequence DNA. Also linear dichroism indicates that a change in binding geometry occurs at high polyamide/AT ratios, and that the effect occurs only with polyamide in contrast to DST. Since the effect is insignificant with DST, or with calf thymus DNA, it is concluded that it relates to the sizes of the ligands and the minor grooves, becoming critical in the limit of crowding.

INTRODUCTION

Earlier strategies for gene targeting have been based mainly on major groove binding (triplex) or on duplex formation by strand displacement (such as by PNA) (1,2). However, minor groove binding can be anticipated to be a more forceful strategy to target chromatin DNA (3). Here, hairpin polyamides are particularly interesting, a class of synthetic DNA-binding molecules chemically related to the natural product distamycin A. They were invented by Peter Dervan and coworkers with the aim of designing nucleo-basepair specific agents recognizing gene targets via binding in the minor groove of double-stranded DNA, with affinity comparable to DNA binding proteins (4–6). To target DNA via the minor groove is thus an interesting alternative, as recently successfully demonstrated by arresting the proliferation of cancer cells using a polyamide alkylator chosen after screening a small library of compounds (7). Such a targeting strategy is not restricted to a single target, but could involve binding to accessible mismatched targets, when the match target might be shielded by histone binding or be less accessible due to kinetic factors. Therefore, more knowledge is desirable on the binding mechanism, affinity, and dynamics of DNA complexes with the Dervan polyamides, also regarding their nonspecific DNA complexes occurring at higher ligand/DNA ratios.

The hairpin polyamides consist of two chains of heterocyclic amides, linked by a four-amino butyric acid group (γ), allowing the molecule to fold back upon itself with two planar, aromatic ring systems stacked side-by-side in an antiparallel orientation within the minor groove. The sequence specificity is determined mainly by hydrogen-bonding and steric fit in the minor groove by the sequence of side-by-side pairings of aromatic rings of the polyamide ligand. Here *N*-methylimidazole (Im) is specific for guanine; *N*-methylpyrrol (Py) binds to adenine, cytosine, and thymine, whereas *N*-methylhydroxypyrrole (Hp) is specific for thymine (8–13). The structure of each of these rings in position with its corresponding basepair in oligonucleotides has been characterized by NMR and by x-ray structure analysis, revealing a pattern of specific hydrogen bonds between polyamide and the edges of the Watson-Crick basepairs, as well as an ensemble of van der Waals contacts between the polyamide and the walls of the minor groove (11,14). Footprinting and affinity cleaving studies have provided a large amount of information regarding the DNA sequence affinities and orientations of Im-Py polyamides (15).

Comparing the binding of the polyamide to that of distamycin A (DST, in polyamide notation Py-Py-Py) may provide further insight into the mechanisms of minor groove recognition. There have been numerous studies characterizing DST-DNA complexes; DST is reported to bind to DNA with two modes of binding, a 1:1 motif and a 2:1 binding mode (10,16,17). X-ray crystallographic and NMR studies have indicated a binding preference for stretches of A (or T) bases by the 1:1 complex. However, the 2:1 complex, which is structurally more similar to the polyamide-DNA complexes, appears to prefer sequences with more A-T alterations, which are known to allow for a wider minor

Submitted January 2, 2006, and accepted for publication April 25, 2006.

Address reprint requests to Bengt Nordén, Tel.: 46-31-7723041; E-mail: norden@chalmers.se.

0006-3495/06/08/904/08 $2.00

doi: 10.1529/biophysj.105.080564

groove (17–20). Binding sites of at least 5 bp can accommodate two DST molecules side-by-side in an antiparallel orientation (10). Correspondingly, the eight-ring hairpin polyamide, ImPyPyPy-(R)$H_2N\gamma$-ImPyPyPy-β-Dp, may target a DNA sequence of 5′-WGWWCW-3′, where W is either T·A or A·T basepairs (21). The binding site size for an eight-ring polyamide is reported to be 6 bp, determined by footprinting analysis (22). Earlier studies have shown that the polyamide has its highest affinity for the 5′-AGTACT-3′ sequence, with a binding constant of 10^{10} M^{-1} (22).

A recent study using fluorescence melting showed that the polyamide binds to the target sequence and not to the reverse sequence, but also to a sequence containing a central AC mismatch, as well as to an AT_n sequence and to an A_n sequence (23). We were intrigued by the many sequences to which a polyamide could bind, and wanted to investigate whether it binds in the same manner to different kinds of large DNAs. High concentration of polyamide on DNA was studied to evaluate the importance of unspecific binding. Calf thymus DNA provides a model for heterogeneous DNA, representing effectively all sequence combinations, whereas AT-DNA provides the opportunities to study binding to a ''double mismatch'' site and to investigate if the size of the minor groove may affect the mode of binding.

In this study, the polyamide ImPyPyPy-(R)$H_2N\gamma$-ImPyPyPy-β-Dp (Fig. 1) is investigated using polarized light spectroscopy to characterize the binding geometry of the complex with large DNA (calf thymus DNA and AT-DNA). It is important to further characterize the DNA binding modes of the polyamides to understand the basis of their nucleo-base specificity and potential interference with the expression of the targeted genes through binding in the minor groove of DNA. Investigating also the effect of high concentrations of polyamide could be of interest for assessing effects of varied binding geometry and nonspecific DNA interactions. In this context, distamycin A, a well-studied minor groove binder, was considered relevant to include for comparison, and also because this drug is known to exhibit both monomeric and dimeric binding modes. Linear dichroism (LD), i.e., differential absorption of linearly polarized light, was used to diagnose minor groove binding by the angular orientation of the drugs, and circular dichroism (CD) to further characterize their binding modes and mutual proximity of bound drug molecules. To analyze the binding distribution of the ligands relative to DNA, a singular value decomposition (SVD) approach was found useful: applying SVD to a matrix containing the acquired LD and CD spectra provides an estimate of number of spectroscopic species in the solution and allows under favorable conditions also assignment of specific (groove bound) and unspecific binding, respectively. Interestingly, both the polyamide and distamycin were in this way shown to exhibit two modes of binding depending upon the ligand/DNA ratio.

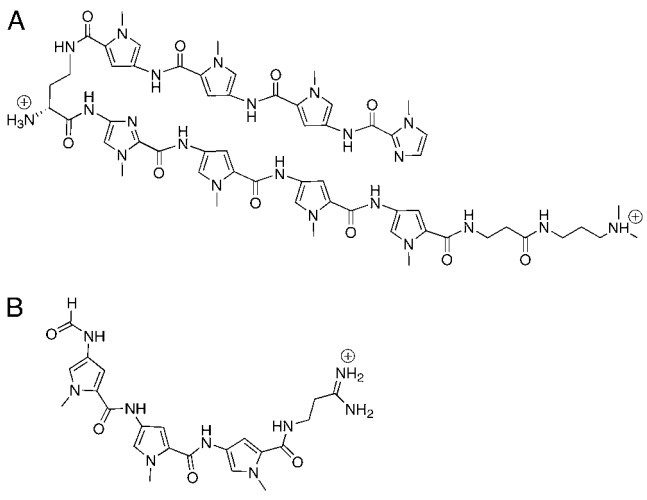

FIGURE 1 Structure of polyamide ImPyPyPy-(R)$N_2H\gamma$-ImPyPyPy-β-Dp (*A*) and Distamycin A (*B*).

EXPERIMENTAL PROCEDURES

Sample preparation

All experiments were performed in 10 mM Tris-HCl, 20 mM NaCl, pH 7.2 buffer. Calf thymus (ct) DNA (highly polymerized, sodium salt) and AT-DNA (poly(dA-dT)·poly(dA-dT)) were purchased from Amersham Pharmacia Biotech, Uppsala, Sweden. Stock solutions were prepared in buffer, filtered, and stored at 8°C. For the LD and CD measurements, the concentration was ranging between 20 and 82 μM bp DNA. The concentration of all duplex nucleic acid samples was confirmed by measuring the absorbance on a Cary 4B spectrophotometer (Varian, Palo Alto, CA), using an extinction coefficient of 6600 $cm^{-1}M^{-1}$ at 260 nm for AT-DNA and at 258 nm for ct DNA.

Polyamides

The polyamide, ImPyPyPy-(R)$H_2N\gamma$-ImPyPyPy-β-Dp (where β is β-alanine and Dp is 1,3-diaminopropan) was prepared manually using solid-phase protocols described elsewhere (24). Starting materials for the pyrrole and imidazole monomers were kindly provided by Professor Peter Dervan (California Institute of Technology). The polyamide was purified and analyzed using high-performance liquid chromatography (purity >95%) and MALDI-TOF mass spectrometry (1237,3 g/mol). Concentration of polyamide was determined spectrophotometrically using an extinction coefficient of 69,500 $cm^{-1}M^{-1}$ at 312 nm at 25°C (24). Distamycin A hydrochloride was purchased from Sigma Aldrich (St. Louis, MO) and used without further purification. Due to the slow hydrolysis of DST in water, stock solutions were freshly prepared in water and used within 2 days. Drug concentration was determined spectrophotometrically, applying a molar absorption extinction coefficient of 35,000 $cm^{-1}M^{-1}$ at 316 nm (25). All measurements were performed at 25°C.

Circular dichroism spectroscopy

CD is defined as the difference in absorbance of left and right circularly polarized light. All CD measurements were performed on a Jasco (Easton, MD) J-720 spectropolarimeter using a 1 cm cell. All spectra were recorded between 220 and 390 nm and corrected for background contributions. The sensitivity and scan speed was set to 1 mdeg and 50 nm min^{-1}, respectively. Three scans were accumulated and averaged by computer. The data were

converted from ellipticity units to absorbance units as: CD (absorbance units) = ellipticity difference (degrees)/33.0.

Flow linear dichroism spectroscopy

LD is defined as the difference in absorption of linearly polarized light and perpendicular polarized light relative a macroscopic orientation axis (flow direction of DNA). LD was measured as described elsewhere using a Couette flow cell and a modified circular dichroism spectropolarimeter (26,27).

The data were converted from ellipticity units to absorbance units as: LD (absorbance units) = ellipticity difference (degrees)/33.0. The reduced linear dichroism LD^r is calculated as

$$LD^r = LD(\lambda)/A_{isotropic}(\lambda), \quad (1)$$

where $A_{isotropic}$ is the absorption of the corresponding isotropic sample. The LD^r of a single electronic transition in a uniaxially oriented sample can be written as

$$LD^r = S\left(\frac{3}{2}\right)(3\cos^2\alpha - 1) \quad (2)$$

where α is the angle between the transition moment and helix axis of DNA and S is an orientation factor describing the average degree of orientation of the helix axis relative to the flow coordinate system ($0 \leq S \geq 1$).

Singular value decomposition analysis

SVD allows a model-free determination of the number of linearly independent components in a given matrix of data. Any $m \times n$ matrix, $\mathbf{A}$ ($m \geq n$), can be written as the product of an $m \times n$ column-orthonormal matrix $\mathbf{U}$, an $n \times n$ diagonal matrix with nonnegative elements, $\mathbf{S}$, and the transpose of an $n \times n$ orthonormal matrix $\mathbf{V}$: $A = \mathbf{U} \times \mathbf{S} \times \mathbf{V}^T$. For examples, when $\mathbf{A}$ is a collection of spectra of samples, each spectrum is a column in $\mathbf{A}$. The $\mathbf{U}$ matrix contains the wavelength-dependence of the different orthogonal ''species'' contained in matrix $\mathbf{A}$; the diagonal of the $\mathbf{S}$ matrix gives the weight of each species as singular values $\sigma_1 \geq \sigma_2 \geq \ldots \geq 0$ and the $\mathbf{V}$ matrix gives the variation in intensity of the different ''species'' in the samples. SVD analysis can determine the number of significant binding components when titrating ligand into a DNA solution. SVD was performed using MATLAB 6.5 computational software (The MathWorks, Natick, MA) (28). Briefly, the SVD analysis on LD and CD data was performed accordingly: the LD and CD data were scaled to the same DNA concentration, followed by scaling the LD to the CD max value. The MATLAB command [u,s,v] = svd was employed to a data set that was incrementally expanded by including all spectra of the series. The data set was restricted to longer wavelengths than 300 nm, due to spectral overlap between DNA and the ligands. The binding isotherms are presented as log σ.

RESULTS

Circular dichroism of polyamide-DNA complex

The polyamide is formally chiral due to the γ-linker but free in solution, as a result of conformational flexibility, it does not exhibit any significant CD. Upon binding to ct DNA or AT-DNA, a relatively strong induced CD is observed (Fig. 2). At low binding ratios, the CD spectrum has several isodichroic points (ct DNA: 250, 261, and 311 nm; AT- DNA: 250, 267, and 313 nm), consistent with effectively two spectroscopic species. At a drug/DNA ratio of 0.16, the CD spectrum exhibits an exciton-like bisignate feature and is red shifted, in both ct and AT-DNA, indicating interactions between bound drug molecules.

To quantify characteristics of the collected CD spectra, SVD was employed. The experimental CD spectra were treated as a set of vectors, and from those data alone, without imposing additional constraints, SVD was used to find an orthogonal set of ''subspectra'' that could be linearly combined to construct the original set of spectra. The linear coefficients are given as singular values, σ. These correspond to the orthogonal species that are necessary, in principle, to describe all spectra. One binding mode would produce just one species in the SVD analysis. Two singular value vectors (σ_1 and σ_2) were found to be markedly more important than the remainder, suggesting that the binding process involves two different bound species in both ct and AT DNA.

The growth of the primary (σ_1) and the secondary singular values (σ_2) are very similar to each other both with ct and AT-DNA, the difference being that the secondary binding mode in the SVD analysis, including all spectra, contributes more to the overall spectra in AT compared to ct DNA. The SVD analysis of the primary species gives a binding site size of ~5 bp for the polyamide.

Flow linear dichroism of polyamide-DNA complex

The polyamide in buffer does not itself show any LD signal, as it is too small to become significantly aligned by the flow field, but does exhibit LD upon binding to the flow-oriented DNA. The intensity and sign of the LD signal is dependent on the orientation of the drug relative to the direction of the orientation axis of DNA (the helix axis). The polyamide has a positive LD for the long-axis polarized absorption band at 300 nm, in its complex both with ct and AT-DNA (Fig. 2). Due to strong overlap between the ligand absorption with the DNA absorbing region, the orientation factor S was gauged in absence of ligand using the LD^r of the nucleotide band at 260 nm, assuming an average α of the nucleobases of 86° (26). S was determined to 0.14 and 0.018 for ct and AT DNA, respectively, giving an α of 44° ± 3° for the polyamide both with ct and AT-DNA (Fig. 3). This angle is not consistent with an intercalative binding geometry but is consistent with groove binding, and is furthermore in close agreement with angles observed for minor-groove binding (26). The LD of polyamide associated to ct DNA shows a roughly linear increase upon addition of ligand up to a ligand/DNA bp molar ratio of 0.3. Although the shape of the spectrum is retained, the intensity decreases with higher ratios. The polyamide-AT complex shows a similar LD as the ct complex at ratios below 0.3. However, at higher ratios, the LD is significantly perturbed, showing instead a very strong negative LD signal, increasing approximately linearly with added amount of ligand. This behavior is readily reversible, the spectra resuming their original appearance upon addition of more AT-DNA to the sample to decrease

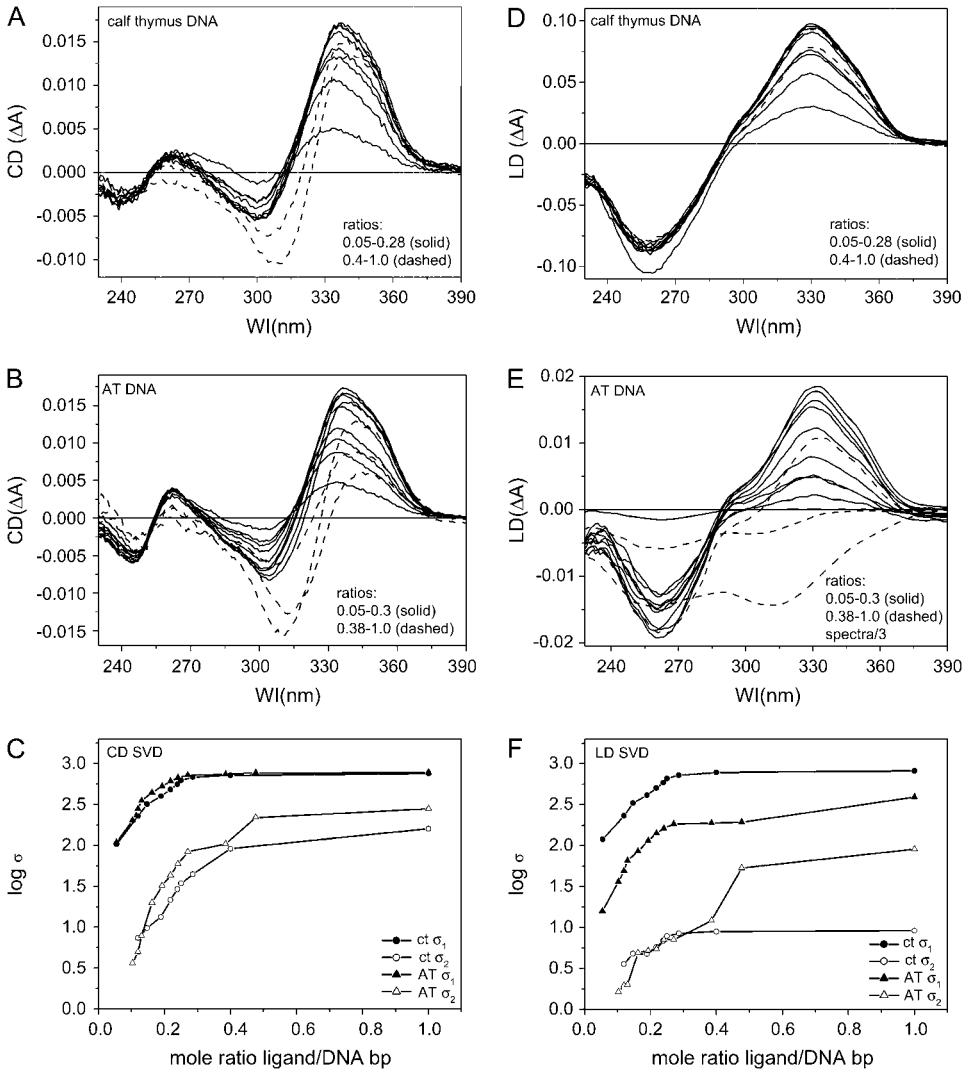

FIGURE 2 (*A*) CD spectra of the polyamide associated to ct DNA (ligand/DNA basepair ratios 0.05–0.28 *solid lines*, ratios 0.4–1.0 *dashed lines*). (*B*) CD spectra of the polyamide associated to AT-DNA (ratios 0.05–0.3 *solid lines*, ratios 0.38–1.0 *dashed lines*). (*C*) SVD analysis of the CD data of polyamide associated to ct DNA and AT-DNA. (*D*) LD spectra of the polyamide associated to ct DNA (ratios 0.05–0.28 *solid lines*, ratios 0.4–1.0 *dashed lines*). (*E*) LD spectra of the polyamide associated to AT-DNA_2 (ratios 0.05–0.3 *solid lines*, ratios 0.38–1.0 *dashed lines*). (*F*) SVD analysis of the LD data of polyamide associated to ct DNA and AT-DNA (first singular species (σ_1) *solid circles*, second singular species (σ_2) *open circles* for ct DNA and first singular species (σ_1) *solid triangles* (7), second singular species (σ_2) *open triangles* (8) for AT-DNA).

the ligand/nucleobase ratio. The SVD analysis of the LD data shows that only one binding mode is observed in ct DNA. In AT-DNA, the secondary species, σ_2, is present even at low ligand/DNA bp molar ratios, with a larger contribution to the binding at ratios above saturation. The SVD analysis gives a binding site size of ~4 bp for the polyamide.

Circular dichroism of DST-DNA complex

Like the polyamide, DST does not exhibit any significant CD by itself but becomes optically active upon binding to DNA. Addition of ligand to DNA produces a strong positive-induced CD signal at 300–360 nm, indicative of a specific binding to DNA (Fig. 4). Each spectrum exhibit several isodichroic points (ct DNA: 250, 274, and 307 nm, AT-DNA: 250, 280, and 305 nm). A red shift of the spectra is gradually appearing during the titration, the effect being most pronounced in DST bound to AT-DNA. The SVD of DST-AT complex shows that σ_2 contributes more to the CD in AT compared to ct. The secondary binding mode contributes very little to the overall CD spectra, and gives a binding site size of 5 bp for the distamycin dimer in ct DNA and 6 bp for AT-DNA.

Flow linear dichroism of DST-DNA complex

DST has a positive LD signal in both AT-DNA and ct DNA, in agreement with a minor-groove binding geometry (Fig. 4). The angle was determined to 42° ± 3° in both DNA samples, thus within experimental error identical to the angle observed for the polyamide, and an angle typical for minor-groove binding (26). For both types of duplex, the shape of the LD spectrum is retained throughout the titration. For ct DNA, the positive LD signal continues to increase also at ratios above 0.25. By contrast, for AT-DNA, the initial increase is followed by a substantial decrease in LD signal at ratios above the proposed binding site size of 5 bp. In contrast to the case for the polyamide, the SVD analysis of

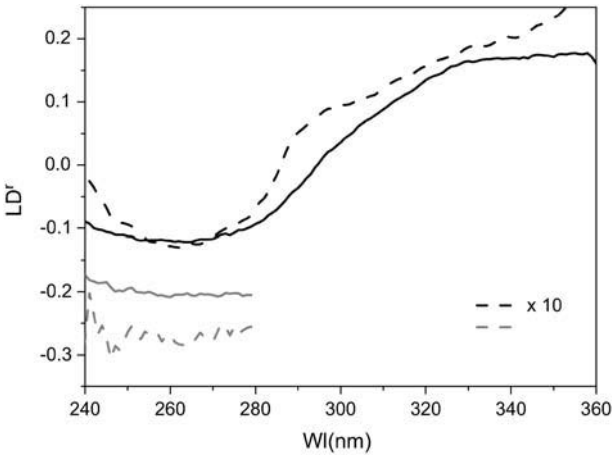

FIGURE 3 LD^r of polyamide: calf thymus complex (*solid line*) and polyamide-AT DNA complex (*dashed line*, magnified ×10). Ratio of 0.2 polyamide/duplex. $S = 0.14$ and 0.018 for ct DNA (*shaded solid line*) and AT DNA (*shaded dashed line*, magnified ×10), respectively.

the LD data for DST suggest only one mode of binding, σ_2, contributing very little to the overall LD spectra. The SVD analysis gives a binding site size of 5 bp for the distamycin dimer in ct DNA and 6 bp for AT-DNA.

DISCUSSION

The prospect of using minor groove binding ligands as nucleo-base specific agents that interfere with the expression of the targeted genes necessitates an understanding and a characterization of their binding behavior. Important clues regarding the nature of DNA interaction can be obtained in studies using polarized light, combined with singular value decomposition analysis. This article describes a comparative study of the interactions of a hairpin polyamide with large DNA and the minor groove binder distamycin. Specific, as well as nonspecific, binding of the ligands has been investigated.

In our study, the polyamide exhibits, as judged from affinity as well as binding geometry in a region of intermediate and higher ligand/DNA ratios, no marked sequence discrimination between mixed-sequence and AT DNA, but shows similar binding affinity to both ct and AT-DNA. Furthermore, the experiments show that two different binding modes are present in both DNA contexts depending upon ligand/DNA ratio.

The results obtained from the CD measurements show interesting differences and similarities between the two ligands investigated. Due to their structural relationship, common binding features were expected. The well-characterized DST exhibits CD spectra in agreement with what has been reported in the literature. Chen and Sha (29) concluded four CD characteristics for dimer binding: i), maintenance of the isodichroic point at 240 nm, ii), a red shift and intensity increase in the 325 nm band, iii), the appearance of a negative indentation at 291 nm, and iv), the presence of additional isodichroic points at 284 nm and 306 nm. Comparing the CD data (Fig. 4) with these criteria, what is observed throughout the titration is the 2:1 DST complex in ct and AT-DNA. Similar characteristics are also observed for the polyamide (Fig. 2), suggesting that the hairpin conformation is maintained through the titration. The exciton-like bisignate feature accompanied by the red shift observed for the polyamide-duplex complexes are not observed to the same extent for DST. On the contrary, the DST binding does not appear as prone to crowding as the polyamide, as supported by both the CD and LD data.

Interestingly, polyamide bound to ct DNA and AT-DNA reach the same maximal CD signal, indicating that the polyamide does not effectively distinguish between ct DNA and AT-DNA at these high overall concentrations, but shows considerable sequence tolerance in the limit of high saturation. To quantify characteristics of the collected CD spectra, SVD was employed. The SVD of the DST CD shows that a second binding mode is only observed for the DST-AT complex at ratios above saturation, producing a rather distinct red shift, accompanied by a reduction in the intensity of the 325 nm band. The CD of the polyamide shows an exciton-like red shift at high ratios, for both duplexes, very similar to the DST-AT complex. The SVD of the polyamide CD shows that a second binding geometry, different from that of the groove bound hairpin mode, contributes to the CD spectra, even at ratios below saturation. The existence of two SVD components is strong evidence for two distinct binding modes. The secondary binding mode for the polyamide-AT complex contributes more to the overall CD spectra compared to that of ct DNA. The increase of the secondary species with increasing ligand/DNA bp ratio indicates that the CD is particularly sensitive to bimolecular interactions between polyamide molecules, producing an excitonic effect. At visual inspection the CD spectrum changes markedly first at very high ratios; however, the SVD analysis reveals that a second binding mode is present already at very low ligand ratios.

AT and ct DNA have structural differences as well as sequential. The size of the minor groove in AT DNA compared to ct DNA is much more narrow. The exciton effect of the polyamide is largest in AT DNA, which is consistent with close packing of ligands in the minor groove. A possible explanation is that the size of the groove influences the binding mode of the polyamide at high saturation, producing the observed spectral changes. Hairpin polyamides, as well as DST, are reported minor groove binders, and the results obtained in this study clearly support this mode of binding. Both ligands show induced CD upon binding to either duplex, due to interactions with the transition moments of the chirally organized surrounding nucleobases and potentially also due to a selection of a chiral conformation of the skewed

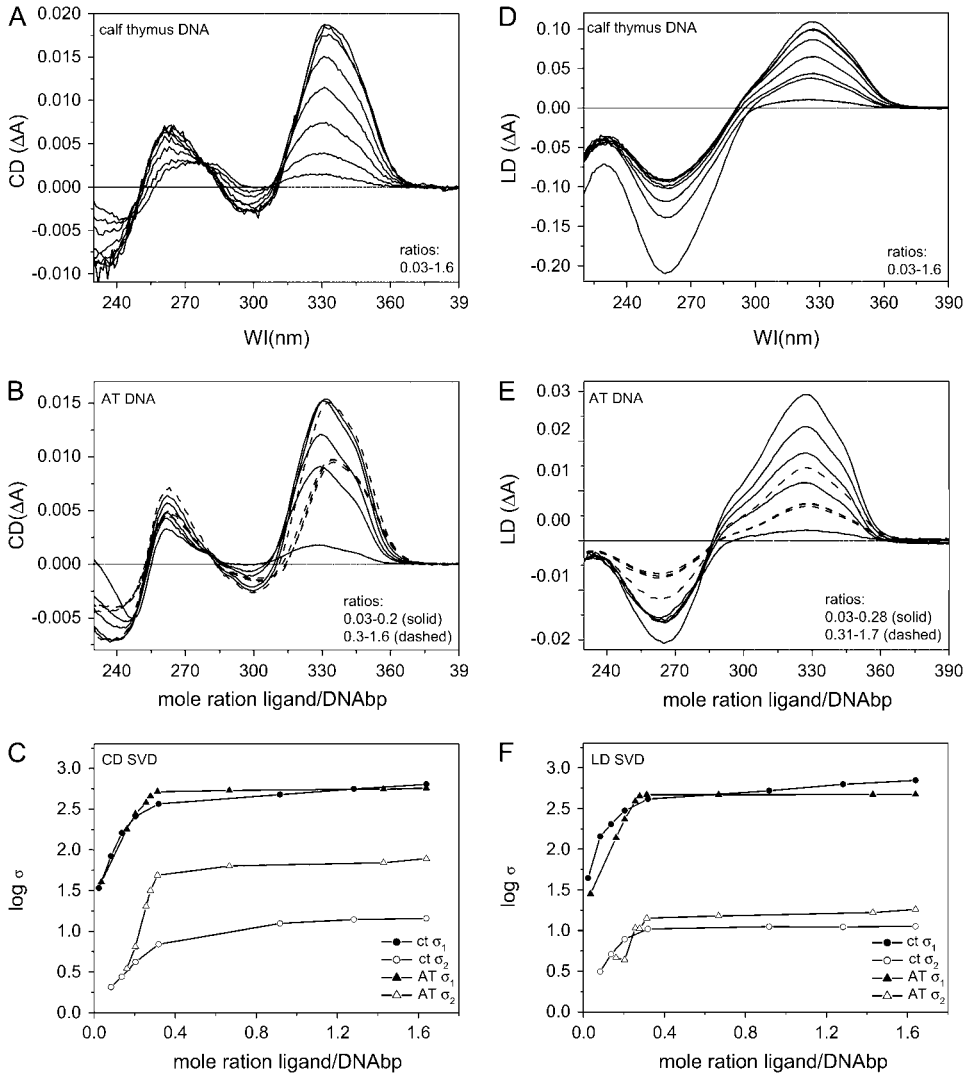

FIGURE 4 (*A*) CD spectra of DST associated to ct DNA. (*B*) CD spectra of DST associated to AT-DNA (ratios 0.03–0.28 *solid lines*, ratios 031–1.7 *dashed lines*). (*C*) SVD analysis of the CD data of DST associated to ct DNA and AT-DNA. (*D*) LD spectra of DST associated to ct DNA. (*E*) LD spectra of DST associated to AT-DNA (ratios 0.03–0.28 *solid lines*, ratios 031–1.7 *dashed lines*). (*F*) SVD analysis of the LD data of DST associated to ct DNA and AT-DNA (first singular species (σ_1) *solid circles*, second singular species (σ_2) *open circles* for ct DNA and first singular species (σ_1) *solid triangles* (7), second singular species (σ_2) *open triangles* (8) for AT-DNA).

ligand upon binding of the polyamide to DNA. Molecules that bind in the minor groove of DNA typically exhibit a strong induced positive CD band for transitions polarized parallel to the groove due to coupling between long-axis polarized ligand transitions and in-plane nucleobase transitions (30). The polyamide as well as DST both exhibit positive induced CD at their long-axis polarized transitions and are thus very probably binding to DNA in such a manner. Analysis of the LD data confirms that the polyamide and DST are groove binders both in ct and AT-DNA. The positive LD is in agreement with an orientation of the long-axis parallel to the groove (at ~45° to the helix axis), a geometry expected with minor-groove binding (26). One could argue that the CD and the positive LD alone cannot completely rule out major groove binding. However, NMR and x-ray crystallography both support minor groove binding of the polyamide (4–6,11,14) and of DST (10,16–19). Taken together with the transition moment angle (~45°) being in perfect agreement with a snug fit in the minor groove in the wide range of binding ratios investigated supports minor groove binding. Both ligands have very similar LD spectra, for both ct and AT-DNA, with the exception for the polyamide-AT complex in the limit of saturation. In ct DNA, the shape of the LD spectrum is conserved throughout the titration, in correlation with that, LD detects a single mode of binding only. This is further supported by the SVD analysis for both DST and the polyamide, having a negligible secondary species in ct DNA. This could suggest that the bound polyamide molecules are closely packed along the DNA in the minor groove, thus producing an exciton effect in CD, but not changing the average orientation angle of the overall bound polyamide, explaining the invariant shape of the LD spectrum.

Interestingly, the polyamide-AT DNA complex shows a distinct secondary binding motif according to the SVD analysis. The polyamide-AT DNA complex exhibits a different LD compared to that of DST. The LD being positive at low ratios, as expected for a minor groove binding geometry,

changes to strongly negative at high ratios. An accompanying increase in overall intensity is most likely an effect of increased degree of orientation due to a stiffening of the DNA helix when saturated with ligands in the groove. Since the process is momentarily reversible, no denaturation or higher-order structural reorganization of the DNA has occurred at high ligand ratios. A strongly negative LD is normally indicative of an intercalative binding mode; however, the length of the ligand appears incompatible with such a binding mode unless considerable conformational changes take place. The observation that only AT DNA is affected in such a manner in the presence of high amount of polyamide indicates that the effect be related to the nucleobase-ligand interaction or to that the size of the minor groove (more narrow in AT context) influences the binding orientation of the bound polyamide molecules in the limit of saturation. The latter explanation is in accord with the DNA helix becoming more rigid and well oriented by the flow.

CONCLUSIONS

The most important finding in this work is that both the polyamide and DST DNA ligands display two modes of binding in a manner that depends on the ligand/DNA basepair ratio. We report that the hairpin polyamide binds strongly to both mixed-sequence and AT DNA, with little binding-geometric differences at low ligand/DNA ratios. At high binding ratios, distinct differences between the duplexes are observed: CD excitonic effects provide evidence for bimolecular interactions of the polyamide in the case of AT DNA, and LD reveals a change in binding mode occurring at very high polyamide-AT ratios. Since the effect is not observed for mixed-sequence (ct) DNA, or with the smaller DST ligand, it is concluded to be a result of the size of the minor groove being important in the limit of crowding.

We thank Professor Peter Dervan for kindly providing starting material for the polyamide synthesis.

This work was supported by the European Commission.

REFERENCES

1. Egholm, M., O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kims, B. Norden, and P. E. Nielsen. 1993. PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen-bonding rules. *Nature.* 365:566–568.
2. Franscois, J. C. 1988. Sequence-specific recognition via the major groove of DNA by oligodeoxynucleotides via triple helix formation. *Nucleic Acids Res.* 16:11431–11440.
3. Suto, R. K., R. S. Edayathumangkan, C. L. White, C. Melander, J. M. Gottesfeld, P. B. Dervan, and K. Luger. 2003. Crystal structure of nucleosome core particles in complex with minor groove DNA-binding ligands. *J. Mol. Biol.* 326:371–380.
4. Dervan, P. B. 2001. Molecular recognition of DNA by small molecules. *Bioorg. Med. Chem.* 9:2215–2235.
5. Dickinson, L. A., R. J. Gulizia, J. W. Trauger, E. E. Baird, D. E. Mosier, J. M. Gottesfeld, and P. B. Dervan. 1998. Inhibition of RNA polymerase II transcription in human cells by synthetic DNA-binding ligands. *Proc. Natl. Acad. Sci. USA.* 95:12890–12895.
6. Gottesfeld, J. M., J. M. Belitsky, C. Melander, P. B. Dervan, and K. Luger. 2002. Blocking transcription through a nucleosome with synthetic DNA ligands. *J. Mol. Biol.* 321:249–263.
7. Dickinson, L. A., R. Burnette, C. Melander, B. S. Edelson, P. S. Arora, P. B. Dervan, and J. M. Gottesfeld. 2004. Arresting cancer proliferation by small-molecule gene regulation. *Chem. Biol.* 11: 1583–1594.
8. Geirstanger, B. H., M. Mrksich, P. B. Dervan, and D. E. Wemmer. 1994. Design of a G.C specific DNA minor groove-binding peptide. *Science.* 266:646–650.
9. Kielkopf, C. L., S. White, J. W. Szewczyk, J. M. Turner, E. E. Baird, P. B. Dervan, and D. C. Rees. 1998. A structural basis for recognition of A.T and T.A base pairs in the minor groove of B-DNA. *Science.* 282:111–115.
10. Pelton, J. G., and D. E. Wemmer. 1989. Structural characterization of a 2:1 distamycin A:d(CGCAAATTGGC) complex by two dimensional NMR. *Proc. Natl. Acad. Sci. USA.* 86:5723–5727.
11. Pelton, J. G., and D. E. Wemmer. 1990. Binding modes of Distamycin A with $d(CGCAAATTTGGG)_2$ determined by two-dimensional NMR. *J. Am. Chem. Soc.* 112:1393–1399.
12. Wade, W. S., M. Mrksich, and P. B. Dervan. 1992. Design of peptides that bind in the minor groove of DNA at 5′-(A,T)G(A,T)C(A,T)-3′ sequences by a dimeric side-by-side motif. *J. Am. Chem. Soc.* 114: 8783–8794.
13. White, S., J. W. Szewczyk, J. M. Turner, E. E. Baird, and P. B. Dervan. 1998. Recognition of the four Watson-Crick base pairs in the DNA minor groove by synthetic ligands. *Nature.* 391:468–471.
14. Mrksich, M., J. C. Watkins, T. J. Dwyer, B. H. Geirstanger, P. Weyermann, and P. B. Dervan. 1992. Antiparallel side-by-side dimeric motif for sequence-specific recognition in the minor groove of DNA by the designed peptide 1-methylimidazole-2-carboxamide netropsin. *Proc. Natl. Acad. Sci. USA.* 89:7586–7590.
15. White, S., E. E. Baird, and P. B. Dervan. 1997. Orientation preferences of pyrrole-imidazole polyamides in the minor groove of DNA. *J. Am. Chem. Soc.* 119:8756–8765.
16. Chen, X., B. Ramakrishnan, and M. Sundaralingam. 1997. Crystal structures of the side-by-side binding of distamycin to AT-containing DNA octamers d(ICITACIC) and d(ICATATIC). *J. Mol. Biol.* 267: 1157–1170.
17. Coll, M., C. A. Frederic, A. H.-J. Wang, and A. Rich. 1987. A bifurcated hydrogen-bonded conformation in the d(A.T) base pairs of the DNA dodecamer d(CGCAAATTTGCG) and its complex with distamycin. *Proc. Natl. Acad. Sci. USA.* 84:8385–8389.
18. Fagan, P. A., and D. E. Wemmer. 1992. Cooperative binding of distamycin A to DNA in the 2:1 mode. *J. Am. Chem. Soc.* 114:1080–1081.
19. Pelton, J. G., and D. E. Wemmer. 1988. Structural modeling of the distamycin A-$d(CGCGAATTCGCG)_2$ complex using 2D NMR and molecular mechanics. *Biochemistry.* 27:8088–8096.
20. Yoon, C., G. G. Privé, D. S. Goodsell, and R. E. Dickerson. 1988. Structure of an alternating-B DNA helix and its relationship to A-tract DNA. *Proc. Natl. Acad. Sci. USA.* 85:6332–6336.
21. Kelly, J. J., E. Baird, and P. B. Dervan. 1996. Binding site size limit of the 2:1 pyrrole-imidazole polyamide-DNA motif. *Proc. Natl. Acad. Sci. USA.* 93:6981–6985.
22. Trauger, J. W., E. E. Baird, and P. B. Dervan. 1996. Recognition of DNA by designed ligands at subnanomolar concentrations. *Nature.* 382:559–561.
23. James, P. L., L. Le Strat, U. Ellervik, C. Bratwal, B. Nordén, T. Brown, and K. R. Fox. 2004. Effects of a hairpin polyamide on DNA melting: comparison with distamycin and Hoechst33258. *Biophys. Chem.* 111: 205–212.
24. Baird, E. E., and P. B. Dervan. 1996. Solid phase synthesis of polyamides containing imidazole and pyrrole amino acids. *J. Am. Chem. Soc.* 118:6141–6146.

25. Bourdouxhe, C., P. Colson, C. Houssier, J.-S. Sun, T. Montenay-Garestier, C. Hélène, C. Rivalle, E. Bisagni, M. J. Waring, J.-P. Hénichart, and C. Bailly. 1992. Binding of a distamycin-ellipticine hybird molecule to DNA and chromatin: spectroscopic, biochemical, and molecular modeling investigations. *Biochemistry*. 31:12385–12396.

26. Nordén, B., M. Kubista, and T. Kurucsev. 1992. Linear dichroism spectroscopy of nucleic acids. *Q. Rev. Biophys.* 25:51–170.

27. Nordén, B., and S. Seth. 1985. Critical aspects of measurement of circular and linear dichroism: a device for absolute calibration. *Appl. Spectrosc.* 39:647–657.

28. Anderson, E., Z. Bai, C. Bischof, S. Blackford, J. Demmel, J. Dongarra, J. Du Croz, J. Greenbaum, S. Hammarling, A. McKenney, and D. Sorensen. 1999. LAPACK User's Guide, 3rd ed. SIAM, Philadelphia.

29. Chen, F. M., and F. Sha. 1998. Circular dichroic and kinetic differentiation of DNA binding modes of distamycin. *Biochemistry*. 37: 11143–11151.

30. Lyng, R., T. Hard, and B. Nordén. 1987. Induced CD of DNA intercalators: electric dipole allowed transitions. *Biopolymers*. 26: 1327–1345.