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Minor groove to major groove, an unusual DNA sequence dependent change in bend directionality by a distamycin dimer

Shuo Wang, Manoj Munde, Siming Wang, and W. David Wilson*

Department of Chemistry, Georgia State University, Atlanta, Georgia 30303, USA

Abstract

DNA sequence dependent conformational changes induced by the minor groove binder, distamycin, have been evaluated by polyacrylamide gel electrophoresis. The distamycin binding affinity, cooperativity and stoichiometry with three target DNA sequences that have different sizes of alternating AT sites, ATAT, ATATA and ATATAT, have been determined by mass spectrometry and surface plasmon resonance to help explain the conformational changes. The results show that distamycin binds strongly to and bends five or six AT base pair minor groove sites as a dimer with positive cooperativity, while it binds to ATAT as a weak, slightly anti-cooperative dimer. The bending direction was evaluated with an in phase A-tract reference sequence. Unlike other similar monomer minor groove binding compounds, such as netropsin, the distamycin dimer changes the directionality of the overall curvature away from the minor groove to the major groove. This distinct structural effect may enable designed distamycin derivatives to have selective therapeutic effects.

Natural and synthetic heterocyclic cations that bind into the DNA minor groove have demonstrated effectiveness as therapeutic agents as well as probes for use to extend our fundamental understanding of DNA molecular recognition (1–14). Crystal structures with a variety of minor groove binding compounds have significantly extended our understanding of the structural varieties of these systems, the importance of solvent molecules in the complexes and the induced fit effects for binding of both DNA and the bound small molecule (1, 15–22). There is significant recent progress in the development of minor-groove-targeted therapeutics for cancer (9, 23, 24), parasitic diseases (25), and bacterial infections (26, 27), and this is an area of intense current research interest. Topics of specific importance in this research are development of a greater variety of cell-permeable minor groove agents that have increased DNA binding sequence selectivity. Heterocyclic diamidines that target minor groove AT sequences in parasite mitochondrial kinetoplast DNA have shown excellent clinical activity in humans (4, 25). Polyamides based on the natural products, netropsin and distamycin (Dst), have been developed with much broader sequence recognition capabilities (2, 11, 28, 29) than most minor groove binders. The polyamides also have shown cell uptake potential, depending on the polyamide composition, cell types, and conditions (30–32).

The successful sequence recognition development of the polyamides began with the discovery by Wemmer and coworkers (3, 33, 34) that distamycin could bind to some AT minor groove DNA sequences as a stacked dimer and to others as a monomer. NMR (35) and X-ray (36) structural studies of Dst-DNA complexes show that the polyamide can bind

*To whom correspondence should be addressed. Tel: +1 404 413 5503; Fax: +1 404 413 5505; wdw@gsu.edu.

SUPPORTING INFORMATION

ESI mass spectra of Dst with target duplex DNA sequences at five charge states, and with a hairpin sequence containing an ATATA site (Figures S1–S4). This material is available free of charge via the Internet at <http://pubs.acs.org>.

to four successive AT base pairs in the 1:1 mode. The binding mode, however, can change to a cooperative 2:1 stacked, antiparallel complex for some binding site sequences (for example, alternating AT base pair sequences) that contain five or six AT base pairs (33, 37). The extensive, repeated AT sequences in parasite kinetoplast DNA makes such novel AT recognition an attractive development area for improved selectivity in parasite targeting (7, 25). As noted above, the extensive development of the Dst heterocyclic units to design compounds that can recognize a wide variety of different DNA base pair sequences and length, by Dervan, Sugiyama, Lee (2, 11, 28, 29) and coworkers, greatly increases the importance of this chemical group.

We have recently observed that many minor-groove-binding agents cause large conformational changes on binding to alternating AT sequences and such changes may be important for their mode of antiparasitic activity (38, 39). NMR data from the Wemmer group shows that the dimer binding mode of Dst with ATATA has increased cooperativity over binding to AAAAAA (40). These results suggested that the cooperative dimer binding mode of Dst could induce significant changes in the local structure of AT sequences. Surprisingly, the effects of this well-studied and important minor groove binding polyamide on DNA structures have not been systematically evaluated. Such an evaluation along with the unique features of Dst binding as both a monomer and a dimer can help answer the question of how the conformational changes induced by minor groove dimer complexes are distinct from those caused by monomers. This question has been addressed through several powerful methods: polyacrylamide gel electrophoresis (PAGE) with a ligation ladder assay, which is a very sensitive and well established approach for detecting conformational changes in DNA that are induced by minor groove binders (38, 39, 41, 42); mass spectroscopy of Dst complexes with alternating DNA sequences as a function of AT site length and, biosensor-surface plasmon methods. The results provide new insight into the Dst complexes at AT binding sites and show that the 2:1 cooperative binding of Dst induces significant bending effects in the DNA helix. The large bending by the Dst dimer is directed towards the major groove and differentiates Dst from minor groove monomer binders that typically cause bending of DNA sequences towards the minor groove (38). This difference may enable Dst derivatives to have different therapeutic mechanisms than other similar minor groove binding drugs and provides important information for the development of specific inhibitors for transcription factors.

MATERIALS AND METHODS

DNA-Binding Compounds

Dst and netropsin were purchased from Sigma-Aldrich (St Louis, MO, USA), and were prepared as 1 mM stock solutions in double distilled water and kept frozen at -4°C away from light. Purity was verified by NMR and mass spectrometry.

DNA Oligonucleotides

Three 21 base pair duplexes with either four, five or six base pair alternating AT sites (ATAT, ATATA and ATATAT in Figure 1), and two 21 base pair duplexes with two five base pair AT sites with A tracts (AAAAA and mixed A5-ATATA in Figure 1) were used in this study. The standard marker, M21, is also a 21-mer sequence with the same base composition as the five AT base pair test duplexes but lacking AT binding sites (38). An additional marker, a 20 base pair ladder (Bayou Biolabs, Metairie, LA, USA), is a commercially available standard sequence with a double intensity band at 100 base pair for reference. Lyophilized DNA oligomers were purchased from Integrated DNA Technologies, Inc. (IDT, Coralville, IA, USA) with HPLC purification. Double distilled water was added to the solid DNAs to bring the concentration to approximately 1.0 mM, based on the

reported amount of DNA from IDT. The actual concentrations of these single stranded DNAs were then determined using a Cary 300 UV-visible spectrophotometer (Varian, Walnut Creek, CA, USA) at A_{260} with extinction coefficients calculated by the nearest-neighbor method (43). Complementary strands were then combined in a 1:1 molar concentration ratio and annealed in ligation reaction buffer (New England Biolabs, Ipswich, MA, USA) containing 50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM dithiothreitol and 1 mM ATP.

Ligation Ladders and Gel Electrophoresis

The ligation procedure is as described by Tevis (38) and Hunt (39). Briefly, annealed duplexes were 5' phosphorylated using T4 polynucleotide kinase (New England Biolabs) and then ligated with T4 DNA ligase (New England Biolabs) in the buffer provided. Kinasing reactions were incubated for 30 min at 37 °C followed by enzyme deactivation for 30 min at 65 °C. Ligation reactions in 200 μ L contained 2 μ M DNA with 1200U of ligase and were done at room temperature for 20 min followed by enzyme deactivation for 30 min at 65 °C.

Ligation ladders were separated by 8% native polyacrylamide gels (1.5 mm thick, 20 cm long) prepared from a 40% acrylamide solution (29:1, bisacrylamide: acrylamide; Acros Organic, NJ, USA). Electrophoresis was carried out at 200 V (10 V/cm) for 140 min at 25 °C in a BioRad Protean II $\times$ i gel apparatus with TBE buffer (0.089 M Tris, 0.089 M boric acid, 2.0 mM EDTA, pH 8.3) and a BioRad PowerPac Basic 300. Each sample had a final volume of 20 μ L, which contained the following: 10 μ L of 2 μ M DNA, 4 μ L of 6 $\times$ load dye (Promega, Madison, WI, USA), and the amount of compounds needed for the desired ratios of compound to binding site and double distilled water to bring to the final volume. After electrophoresis, gels were stained with SYBR Gold Nucleic Acid Gel Stain (Invitrogen, Carlsbad, CA, USA) at the concentration and time recommended by the manufacturer. The stained gels were imaged using an UltraLum Omega 10 gD Molecular Imaging System (UltraLum, Claremont, CA, USA).

Calculation of R_L

Data for migration analysis and molecular weight assignments for ligation ladder products were obtained using Image Quant TL (Amersham Biosciences, Piscataway, NJ, USA) and were based on the mobility of the ligated M21 duplex. The relative mobility R_L , where $R_L = L_{\text{apparent}}/L_{\text{actual}}$ (38, 44), was calculated for each ligated multimer. The mobility of each band of standard M21 was determined and correlated with its length in base pairs (L_{actual}) and molecular weight. The mobilities of each band of the target DNAs and the DNA-Dst complexes were determined and correlated to apparent base pair lengths (L_{apparent}) and molecular weights based on the standard values.

Mass Spectrometry

Mass spectrometry (MS) experiments were conducted with a Waters Micromass Q-TOF micro (ESI-Q-TOF, Milford, MA, USA) instrument operating in negative mode. The capillary voltage was set to 2200 V, the sample cone voltage to 30 V, and the extraction cone voltage to 3 V. The source block temperature and the desolvation temperature were set to 70 °C and 100 °C respectively. Since mass spectrometry generally requires DNA sequences dissolved in a volatile salt solution, all the sequences tested with mass spectrometry were desalted three times by dialysis with a 1000 Da cut-off membrane (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) in 0.1 M ammonium acetate (NH_4OAc , Fluka, Sigma-Aldrich, St Louis, MO, USA) buffer in order to remove other salt ions from DNA, and the actual concentrations of the dialyzed DNA were determined as above. Samples containing 5 μ M target DNA sequences and appropriate amounts of Dst in

the desired molar ratios to DNA were prepared in 0.1 M NH₄OAc buffer with the final volume of 100 μ L. The sample was introduced into the ion source by direct injection at 5 μ M/min flow rate. DNA and Dst-DNA complexes were examined in the negative ion mode of the electrospray ionization (ESI) mass spectrometer resulting in deprotonation and anion formation as shown in Equation 1. Gas phase ionization:

With n being the number of protons removed from DNA to give the charge state. After the deprotonation, the multiply charged DNAs are evaluated according to the mass (m) to charge (z) ratios. MassLynx 4.1 software was used for processing the data.

Biosensor-Surface Plasmon Resonance (SPR)

SPR measurements were performed with a four-channel Biacore 2000 optical biosensor system (Biacore Inc.) as previously described. Three 5'-biotin labeled DNA hairpins that contain the target alternating AT binding sites with loop sequence underlined, 5'-GGATATATCGTCTCCGATATATCC-3', 5'-GGATATACGTCTCCGATATATCC-3' and 5'-CGATATCGTCTCCGATATCG-3', were immobilized onto streptavidin-coated sensor chips as previously described (45–47). The experiments were conducted in a cacodylic acid buffer (6.25 pH, 100 mM NaCl and 0.005% P20 surfactant). In a typical experiment, 200 μ L of samples at different concentrations were injected onto the chip surface with a flow rate of 25 μ L/min and a 600 sec dissociation period. The surface was regenerated with a pH 2.5 glycine solution followed by multiple buffer injections. Steady-state analyses were conducted and data were fitted with either a two site model or a one site model ($K_2=0$):

$$r = (K_1 \times C_f + 2 \times K_1 \times K_2 \times C_f^2) / (1 + K_1 \times C_f + 2 \times K_1 \times K_2 \times C_f^2) \quad 2$$

where r represents the moles of bound compound per mole of DNA hairpin duplex, K_1 and K_2 are macroscopic binding constants, and C_f is the free compound concentration in equilibrium with the complex. C_f is fixed by the concentration of compound in the injected solution and is thus constant because the solution is continuously replaced in the flow cell. Binding thus occurs at constant C_f while the free DNA and complex concentrations change. The observed steady-state response, RU_{sat} , at saturation of binding sites divided by the calculated response per bound compound, RU_{cal} , gives the maximum binding stoichiometry (n) of the DNA-compound complex, as shown in Equation 3(47):

$$n = RU_{\text{sat}} / RU_{\text{cal}} \quad 3$$

RESULTS

ESI Mass Spectrometry: Binding Stoichiometry Analysis

ESI mass spectrometry analysis is a well-established method for the study of nucleic acid complexes (48, 49). Based on the mass/charge ratios and the relative peak intensities for binding of Dst with DNA duplexes containing alternating AT binding sites of 4-6 base pairs: ATAT, ATATA and ATATAT (Figure 1), the same DNAs used in the ligation experiments, the binding stoichiometry and cooperativity of Dst can be determined. The ESI mass spectra for free DNAs and Dst-DNA complexes were detected at five charge states ($-Z = 10, 9, 8, 7, 6$) with the -7 and -8 peaks dominant (Figures 2 and S1-S3). Peaks labeled with [1:2], [2:2], [3:2] and [4:2] in the figures represent the ratios of Dst ligands to the two binding sites in the duplex DNAs. The [2:2] and [4:2] labels stand for the 1:1 and 2:1 Dst to DNA binding sites

in the complexes, respectively. The [1:2] and [3:2] ratios correspond to complexes having the two binding sites occupied by one or three (a 1:1 and a 2:1 complex) Dst molecules, respectively. When ATAT was treated with Dst at a 1:1 compound to DNA binding site ratio, the most abundant peaks observed were from the 1:1 complexes, whereas, for ATATA and ATATAT under the same conditions, 2:1 complexes have increased intensity. In the mass spectra of the samples prepared with a 2:1 Dst to DNA binding site ratio, the 2:1 complexes became the most abundant species in all three tested duplexes and were clearly dominant in ATATA and ATATAT complexes. A hairpin DNA with an ATATA site, the same sequence tested in SPR but without the biotin label, was also treated with Dst under the same conditions. The 2:1 complex was the predominant species (Figure S4), which is in agreement with the observation of the corresponding duplex sequence. The similar observations with duplex and hairpin sequences validate the use of hairpin sequences in our SPR studies. We have also made similar observations with duplex and hairpin sequences in ITC and SPR experiments (50).

Surface Plasmon Resonance: Determining Affinity, Cooperativity and Stoichiometry

Figure 3(A-C) shows biosensor SPR sensorgrams of Dst binding to ATAT, ATATA and ATATAT hairpin DNA sequences. The biosensor-SPR method provides detailed information on the affinity, stoichiometry, and cooperativity of DNA interactions (45). As the concentrations of Dst are raised from 1 nM to 1 μ M, the response units (RUs) increase until around 80 nM for ATATAT and ATATA (Figures 3A, B) but significantly higher amounts are required to reach saturation with ATAT (Figure 3C). To evaluate the affinity and cooperativity for the interaction of Dst with the different AT sequences, the RU values at each concentration were determined in the steady-state region, where the on and off rates are equal and there is no mass transfer interference, and are plotted versus the C_f values for Dst, the concentration of the compound in each flow solution (Figure 3G). The fitting results in Figure 3G provide several key pieces of information about the interaction of Dst with different DNAs. First, the predicted RU value per bound compound, based on the amount of the immobilized DNAs on the chip, is approximately 30 RUs, and the observed value at saturation was between 55-60 indicating that two molecules bind at the AT sites at saturation for all three DNAs. The sigmoidal behavior of the binding curves for ATATAT and ATATA indicates that Dst binds as a strong dimer with positive cooperativity (cooperativity factor of 40 for ATATAT and 28 for ATATA (51)). For ATAT the SPR curve is hyperbolic and indicates non-cooperative dimer formation at this site with a lower affinity than for the longer sites. The binding constants for Dst binding to all three DNAs are collected in Table 1. For a clearer comparison of the binding affinity with target sequences the average binding constant K on a per molecule basis of bound Dst, the square root of the product of $K_1 \times K_2$ ($(K_1 \times K_2)^{1/2} = K$), is also reported in Table 1.

Netropsin is a well known minor groove binder which has been suggested to bind as a monomer regardless of the number of AT bases in the binding site(1, 3, 8). The netropsin binding profile was compared to those with Dst with the same DNAs and shows a very different pattern. The observed RUs for netropsin at saturation (Figure 3D-F) with AT sequences are about half of those with Dst (Figure 3A-C) indicating that, as expected, netropsin recognizes these sequences as a monomer as opposed to the Dst dimers. At low concentration, netropsin binds with slow kinetics for all DNA sequences, and it is not possible to reach a steady-state plateau in the experimental time range, which is limited by the injection volume. Even for these low concentration samples, it is possible to fit the curves with a 1:1 binding model to determine predicted steady-state RU values for netropsin for use in Figure 3H. At the higher concentrations of netropsin, the steady state was reached, and these values can be used directly in the RU versus C_f plots along with predicted values. The binding constants determined in this manner are given in Table 1. Netropsin binds with

very high K in the order ATATAT>ATATA>ATAT. Comparison of Figure 3A–F clearly shows that the DNA sequences with five and six AT base pair sites with Dst and all three DNAs with netropsin have relatively slow on and off rates but the rates are much faster with ATAT and Dst.

Gel Electrophoresis: Binding Induced Bending and Directionality

Ligation ladders of the three alternating AT target DNA oligomers without and with Dst are compared using PAGE, as shown in Figure 4A. Each oligonucleotide contains a two-base overhang to facilitate the cohesive end ligation. Dst was incubated with ligated sequences at three concentrations to give 1:1, 2:1 and 4:1 compound to binding site ratios. In Figure 4A, as indicated by the colored arrows, the 189 base pair ligated, linear multimers were used to compare the electrophoretic mobilities of each sequence. They were chosen because the free DNAs and complexes of this size have migrated long enough in the gel to be fully separated from the other ligated multimers, and can display more pronounced conformational features than those of shorter or longer lengths under these conditions. The gel results show that the alternating AT duplexes are relatively straight since their ligated multimers migrated in the gel at the approximate rates of the reference sequence, M21, consistent with the findings of Tevis (38). After binding with Dst ATAT did not show significant mobility changes at any ratios. In contrast, an extremely pronounced retardation in migration could be observed for ATATA and ATATAT upon increasing the added concentration ratio to 4:1. However, as shown in Figure 4B, the extent of retardation did not change when the amount of compound was further increased up to a final ratio of 10:1. In Figure 6C the relative mobility, R_L , is plotted as a function of L_{actual} to compare the mobility changes induced by drug binding. The significantly reduced mobilities and increasing R_L values indicate the DNA sequences containing five or six successive alternating AT bases were remarkably bent upon the dimer formation by Dst in the longer sites.

It has been found that consecutive adenines, A-tract, sequences bend DNA in the direction of the minor groove (38, 52). A mixed sequence containing one AAAAA and one ATATA tract in phase was ligated and used to determine the directionality of Dst induced ATATA bending with AAAAA as an internal bending direction reference. As shown in Figure 5A, the 168, instead of the 189, base pair (Figure 4A) ligated linear DNAs were used to compare the electrophoretic mobilities of each sequence, since the intrinsically curved AAAAA formed minicircles with the 189 base pair multimers. The corresponding relative mobility of each target oligomer was calculated based on the size in base pairs (Figure 5B). Dst induced opposing mobility changes in AAAAA and ATATA as can be seen by a comparison of the mobilities of the 4:1 ratios. The slight increase for AAAAA and a remarkable reduction for ATATA in migration results in similar migration rates for these two Dst complexes. This similarity in mobility illustrates that the curvatures of the bound AAAAA and ATATA are approximately the same. If both sequences are bent in the same direction, then the observed curvature for the bound mixed sequence will be approximately equal to the bound AAAAA or ATATA. However, if ATATA bends in a direction opposite that of AAAAA, the bending effects will be partially canceled out and will result in a straighter overall structure similar to free ATATA. In a striking result in the presence of Dst, the bound mixed sequence in Figure 5A migrated much faster than the free one, and at a rate similar to the free ATATA. This much less curved overall structure illustrates that the direction of the bend of ATATA induced by dimer binding of Dst is opposite that of AAAAA.

DISCUSSION

Dst is a very important minor-groove-binding agent since it was the first compound to have experimental proof for cooperative, stacked dimer formation in five base pair or longer binding sites of DNA (33, 34, 37, 40). It is also one of the few minor groove binding

compounds to show dimer formation in pure AT sites. To develop a better understanding of the Dst dimer formation in AT sites, and its effects on DNA structure, an important step in developing additional AT specific dimers with cell uptake as therapeutic agents, conformational changes induced by Dst binding to AT sequences have been evaluated by using PAGE ligation ladder assays, ESI mass spectrometry of complexes and SPR binding determinations with ATAT, ATATA and ATATAT. These sequences were selected based on the ground-breaking NMR studies that suggested a 1:1 Dst complex is preferred in ATAT, while the five and six base pair sites can form a 2:1 stacked dimer (33, 40). The alternating AT sequences possess a wider minor groove than A-tract sites and this significantly changes their affinity for 1:1 and 2:1 Dst complexes relative to A-tract DNA binding sites (40).

In the ESI mass spectra of Dst with ATATA and ATATAT, at a 2:1 added ratio, the dimer complex is the only one observed (Figure 2), while Dst preferentially forms a monomer complex and a weaker dimer at ATAT sites. A more quantitative picture of Dst complex formation with these sequences was determined with biosensor-SPR technology. Dst shows very interesting differences in binding profile in SPR depending on the number of AT base pairs in the binding site. A strong dimer with positive cooperativity is observed with ATATAT and ATATA while a weaker and non-cooperative dimer forms in ATAT. This is in agreement with mass spectroscopy results which show that the monomer binding of Dst to ATAT is detected as the dominant species when the compound is present at below molar concentrations relative to the DNA sequence, while a 2:1 complex becomes the most abundant species at high molar ratios of Dst to ATAT. The binding features observed in mass spectrometry and SPR of Dst with selected target DNA sequences help to understand the different conformational changes observed in PAGE.

The gel electrophoresis method used here has been validated as a qualitative tool to analyze DNA conformational changes induced by minor groove binders (39). In order to overcome partial dissociation of complexes in the gel experiment, high molar ratios of Dst to DNA binding sites (Figure 4B) were evaluated and the complexes that formed at ratios above 4:1 displayed an identical electrophoretic mobility. This observation shows that once the binding sites are saturated to form the dimer, the DNA structural change is maximized and will not be further affected by added compound. Therefore, a higher ratio of compound to DNA than the binding stoichiometry is necessary to surmount the influence from dissociation of compound in PAGE and to evaluate the full changes induced by the bound compounds. The molar ratio of 4:1 was consequently used in the calculation of relative mobility R_L .

The PAGE results and R_L values in Figures 4 and 5 show that at lower ratios (1:1 and 2:1) of Dst added per binding site, monomer binding of Dst had little effect on DNA conformation, while at higher ratios ($\geq 4:1$) a Dst dimer induced a cooperative change and significantly bent ATATA and ATATAT. In contrast, Dst does not have a detectable effect in the gel on the DNA sequence containing ATAT. As detected by SPR (Table 1) the second binding constant of Dst with ATAT is significantly less than $1 \times 10^7 \text{ M}^{-1}$. A K value in this range indicates that the binding is not strong enough to ensure dimer formation in the gel experiment (39). During the process of electrophoresis, weakly bound ($K < 5 \times 10^6 \text{ M}^{-1}$) compounds can dissociate from the negatively charged DNA sequence and if they have a positive charge, migrate towards the opposite electrode. Consequently, only the monomer binding of Dst reaches a significant amount for ATAT and little change of the ATAT structure can be detected by PAGE. Interestingly, the ATATA sequence is strongly bent by the monomer binding of netropsin and many heterocyclic diamidines (38, 39) while the Dst monomer with ATATA (1:1 and 2:1 ratios) shows only small conformational effects. The same effects are observed with the ATATAT binding sites at lower ratios and at all ratios

with ATAT. The lack of an effect of Dst monomer binding is opposite to that with netropsin which causes a marked bending of ATATA (38). The lack of bending cannot be due solely to the fact that Dst is a monomer since other monomers cause large changes in ATATA structure (39). Dst does bind weakly as a monomer to all alternating AT sequences and it apparently is not able to compress the minor groove of AT sequences in a monomer complex, as with other minor groove binders.

In order to evaluate the directionality of curvature of the ATATA and the bend induced by Dst dimers, a mixed sequence was designed which contains an AAAAA site as a curvature reference that bends DNA towards the minor groove and an ATATA test binding site in phase. This kind of mixed sequence containing a target binding site as well as a reference site has been verified as a useful tool in studies of induced DNA bend directionality by covalent (10) and non-covalent minor groove binders (38). In contrast to the results observed by Tevis (38) with a number of minor groove binding agents, the relatively fast migration rate and the increased R_L value of the Dst bound mixed sequence (Figure 5) reveals that Dst induced bending is directed towards the major groove. Since the bending angles of free AAAAA and ATATA are 18° and 5° respectively (38), and the bound mixed sequence migrates in a similar rate with free ATATA, we estimate the bending angle of bound ATATA with Dst dimer is $15^\circ\sim 20^\circ$. This result shows that the strong dimeric binding of Dst to ATATA not only remarkably bends this slightly curved sequence but also changes the directionality of the overall curvature away from the minor groove, the bend direction observed for monomer minor groove binders, to the major groove. This distinctive conformational effect might enable Dst or other polyamides (20, 21, for example) to act as an allosteric modulator of DNA transcription by altering the shape of the major groove and thus reducing the binding affinity of certain DNA transcription factors. This mechanism is different from the suggested mode of action for current minor groove binding antiparasitic drugs (7).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

Dst	distamycin
PAGE	polyacrylamide gel electrophoresis
ESI	electrospray ionization
MS	mass spectrometry
SPR	surface plasmon resonance
RU	response unit of SPR instrument
ITC	isothermal titration calorimetry

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Figure 2.

ESI mass spectra of Dst with the three target DNA duplex containing two alternating AT sites at two concentration ratios of compound to binding site: 1:1 and 2:1. The label [n: 2] (n=1, 2, 3, 4) represents the two binding sites that have been bound by n ligands of Dst at two charge states ($-z=8, 7$).

Figure 3.

Biosensor-SPR sensorgrams (instrument responses in RU values versus time) for binding of Dst to (A) ATATAT, (B) ATATA and (C) ATAT at 25 °C. The unbound compound concentrations in the flow solutions range from 0.001 μ M in the lowest curve to 1 μ M in the top curve. Experiments were conducted in cacodylic acid buffer with 0.1 M NaCl. Biosensor-SPR sensorgrams for binding of netropsin to (D) ATATAT, (E) ATATA and (F) ATAT. The unbound compound concentrations in the flow solutions range from 0.001 μ M in the lowest curve to 0.1 μ M in the top curve. SPR binding plots for (G) Dst and (H) netropsin in the presence of ATATAT, ATATA and ATAT. The RU values from the steady-state region of SPR sensorgrams are plotted versus the unbound compound concentration.

Figure 4.

(A) 8% (29:1) native PAGE gel of Dst with three target DNA sequences: ATAT, ATATA and ATATAT. Ratios on top refer to compound per binding site of DNA. A 21 base pair marker (M21) and a 20 base pair marker are shown as the migration standards. Colored arrows demonstrate migration changes of target sequences and indicate 189 base pair (bp) ligated linear multimers. (B) 8% (29:1) PAGE gel of Dst with ATATA in seven concentration ratios. Colored arrows indicate 189 base pair multimers. (C) Plot of the relative mobility, R_L , as a function of L_{actual} for the three ligated duplexes in the absence (open shape) and the presence (solid shape) of Dst in the molar ratio of 4:1 compound to DNA.

Figure 5.

(A) 8% (29:1) native PAGE gel of Dst with three target DNA sequences: AAAAA, ATATA and mixed A5-ATATA. Ratios on top refer to compound per binding site of DNA sequence. M21 and a 20 base pair marker are shown as the migration standards. Colored arrows indicate 168 base pair (bp) ligated linear multimers. (B) Plot of R_L as a function of L_{actual} as in Figure 4.

Table 1

Biacore equilibrium binding constants for Dst and netropsin.

DNA sequence	ATATAT	ATATA	ATAT
Dst	$K_1=3.0$; $K_2=38$ $K=10.7$	$K_1=1.7$; $K_2=13$ $K=4.7$	$K_1=0.8$; $K_2=0.3$ $K=0.5$
Netropsin	46	24	21

All K values have been multiplied by 10^{-7} and must be multiplied by 10^7 (M^{-1}) to get the values.