A novel dicationic polyamide ligand binds in the DNA minor groove as a dimer

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Abstract We have investigated DNA binding properties of a dicationic polyamide molecule (GL020924) that has exhibited unique protein displacement and gene regulation activities. Fluorescence, thermal melting and electrospray ionization mass spectrometry experiments showed that the binding stoichiometry of GL020924 is 2:1 to various DNA oligomers with 8–11 contiguous A/T bp. In accordance with those findings, circular dichroism experiments showed GL020924 binds as a partially staggered side-by-side dimer spanning 10–12 bp. These observations and molecular modeling studies demonstrate that the 2:1 GL020924–DNA complex may exhibit a novel form of stacking orientation involving at least partially parallel peptide groups.

Key words: Minor groove binder; Binding stoichiometry; Electrospray ionization mass spectrometry

1. Introduction

Small molecules that interact with the DNA minor groove have potential therapeutic applications in targeting tumor growth, and viral, bacterial, fungal and parasitic infections [1–3]. Extensive thermodynamic and structural studies have demonstrated that shape complementarity is a key factor in governing the recognition in the minor groove by a small molecule (for a recent review, see [4]). The crescent-shaped curvature of a small cationic molecule with appropriate width and thickness will bring about favorable hydrophobic and electrostatic interactions. Recently, there is growing evidence that suggests that minor groove binding, unlike the notion of a lock-and-key interaction, is not merely a rigid body interaction [5,6]. Conformational changes during protein–ligand and protein–DNA interactions have been extensively investigated and are thought to play an important role in molecular recognition [7,8]. Although the canonical B-DNA conformation has generally been regarded as more rigid than most protein conformations, recent work has shown that DNA can adopt different conformations upon ligand binding [7,9]. It has been demonstrated that ligands bound to the minor groove can change the global DNA superhelical structure in the presence of topoisomerase I [10]. On a local structural level, the nuclear magnetic resonance (NMR) structure of the distamycin homodimer bound to 5′-dCGCAAATTGCG-3′ [11] showed that the minor groove has to be widened significantly to accommodate two side-by-side distamycin molecules. More recently, Wilson and co-workers demonstrated that a small non-peptide dication also can dimerize upon binding to the DNA minor groove [12,13].

Here we report DNA binding properties of a novel heterocyclic dication (GL020924) comprised of two netropsin-like moieties linked by a central indole through head-to-head amide bonds (Fig. 1A). Many head-to-head polyamide molecules have been shown to bind to the A/T rich minor groove [14,15]. It was shown that GL020924 specifically inhibited expression of an engineered cyclin D1 promoter in MCF7 breast cancer cells by competing for promoter binding of transcription factors [16]. More recently, we have observed a biphasic displacement by GL020924 of a major groove binding protein, the origin of replication binding protein UL9 of the type I herpes simplex virus [17]. We demonstrate in this paper that GL020924 is the first dicationic polyamide compound that can form 2:1 complexes in the DNA minor groove. Different modes of binding can have different effects on the DNA conformation and, thus, may give rise to the biphasic displacement of UL9. Designing ligands that can distort B-DNA is becoming increasingly important in developing agents that have in vivo activity.

2. Materials and methods

2.1. Materials

The oligodeoxynucleotides (ODNs) were purchased from Biosource (Camarillo, CA, USA) and were purified by high-performance liquid chromatography. Poly(dA–dT)$_2$ and poly(dA)–poly(dT) were purchased from Midland Certified Reagent Company (Midland, TX, USA). GL020924 was synthesized at Genelabs (Dr. C. Roberts, manuscript in preparation) and verified by NMR and mass spectrometry (MS). A different synthetic approach was described previously [15]. All solution experiments were performed in duplicate in HEN buffer (10 mM HEPES, pH 7.2, 0.1 mM EDTA and 10 mM NaCl) unless stated otherwise.

2.2. Binding stoichiometry

Ligand–DNA binding stoichiometry was determined as described previously [17] using a hybridization stabilization assay (HSA) except that a 12 bp duplex ODN FQ12 (**F**dCTTTATTATTTT·dAAAATAA-

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Abbreviations: CD, circular dichroism; ESI-MS, electrospray ionization mass spectrometry; HSA, hybridization stabilization assay; ODN, oligodeoxynucleotide

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TAAAG**Q**) was used with a fluorescein fluorophore (**F**) at the 5′ end on one strand and a dabcyl quencher (**Q**) at the 3′ end on the complementary strand.

2.3. Thermal melting experiments

DNA thermal melting was monitored by UV absorbance at 260 nm using a Cary Bio 100 UV spectrophotometer (Varian Analytical Instruments). Varying amounts of GL020924 were mixed with 5 μM ODN (5′-dCGATTATTAAGC-3′) in HEN buffer with 50 mM NaCl. Temperature was typically varied from 15 to 95°C with a ramp rate of 0.2°C/min. To determine the melting temperature (T_m) where half of the duplex molecules dissociate, the first-order derivatives of the absorption–temperature curve were calculated.

2.4. Electrospray ionization MS (ESI-MS)

The mass spectroscopic data were collected using a Mariner Electrospray Orthogonal Time-of-Flight Mass Spectrometer (PE Biosystems), operated in a negative ion mode. Two complementary 15-mer ODNs (S_1: 5′-dGCCAAATTTAACCGC-3′ and S_2: 5′-dGCGGTTAAATTTGGC-3′) containing eight consecutive A/T bp in the central region, flanked by 3–4 G/C bp, were used. The annealed duplex of 100 μM was incubated with GL020924 at ligand–DNA molar ratios of 0.9 or 2.2 in 60 mM ammonium acetate. Before MS analysis, the solution containing the complexes was diluted four-fold with 30% methanol/water (v/v). Addition of methanol to the final solution was found to be necessary to aid desolvation in the ESI process and, therefore, to enhance MS signals. The final solutions for analysis contained 25 μM duplex and varying amounts of GL020924 in 22% (v/v) methanol/water with 15 mM ammonium acetate. The sample infusion rate to the mass spectrometer was 6 μl/min.

2.5. Circular dichroism (CD)

Binding of GL020924 to sequence homogeneous polymeric DNA (poly(dA–dT)$_2$ and poly(dA)–poly(dT)) was monitored using a Jasco J-600 CD polarimeter. At each ligand concentration, the CD signal was obtained as an average of at least two scans over the range of 200–400 nm. The DNA polymer was kept at 50 μM in bp concentration while the ligand was titrated from 0 to 50 μM.

2.6. Molecular modeling

A molecular model of the putative 2:1 GL020924–DNA complex was built with Insight/Discover (MSI, San Diego, CA, USA) based on the NMR model structure of the distamycin homodimer bound to 5′-dCGCAAATTGCG-3′ (kindly provided by D.E. Wemmer; [11]). Briefly, a DNA 18-mer with 10 consecutive A/T bp was generated by docking two blocks of the 5′-dCGCAAATT-3′ duplex in tandem, adhering to a straight helical axis and reasonable backbone torsion angles at the junction. Two copies of GL020924 were docked into the 5′-CGCAAATTAATTTGCG-3′ construct, such that two pyrrole units plus the amidinium tail could match the equivalent portion of one of the two stacked distamycins. Torsion angles of the remainder of each GL020924 molecule were adjusted to avoid van der Waals clashes.

3. Results and discussion

3.1. Fluorescence HSA and UV thermal melting experiments indicate formation of 2:1 GL020924–DNA complexes

Fig. 1B shows the differences in fluorescence between free FQ12 and GL020924-bound FQ12 at different molar fractions of GL020924. Linear regression showed that the maximum fluorescence change occurred at a GL020924 molar fraction of 0.67 ± 0.07, suggesting that the binding stoichiometry is 2:1 (GL020924:FQ12). Because of the molecular size of GL020924 (spanning 8 bp), it is unlikely that GL020924 can form a stable tandem complex on FQ12. GL020924 did not form a stable complex and exhibited no protection against DNase I cleavage on sequences with only 4 bp of A/T stretches (data not shown; [16]). Therefore, the 2:1 stoichiometry is most consistent with an overlapping dimerization of the ligand in the minor groove. This result is consistent with the 2:1 binding stoichiometry for GL020924 on an 11 bp ODN [17].

Binding of GL020924 greatly increases the DNA melting temperature as shown in Fig. 2. The melting temperature for free DNA (5′-dCGATTATTAAGC-3′) is 34°C, whereas DNA fully complexed with GL020924 has a T_m of 75°C (i.e. $\Delta T_m = 41$°C), indicating a strong affinity towards double-stranded DNA. Upon addition of GL020924 there are two melting transitions centered at 45°C and 75°C, respectively. The transition at 45°C is not as sharp as the high temperature transition, probably due to the proximity to the free DNA transition at 34°C. In fact, at a GL020924 to DNA ratio (R) of 0.5, the transition at 45°C overlaps with the free DNA transition. At $R = 2$, both the transition at 45°C and free DNA transition ($T_m = 34$°C) disappear, indicating that twice the amount of GL020924 is needed to saturate DNA binding. Since concentrations of both GL020924 and DNA are 10–100-fold higher than the dissociation binding constants (data not shown; [17]), the binding event is stoichiometric under our experimental conditions. Therefore, the disappearance of transitions at 34°C and 45°C at $R = 2$ supports a 2:1 binding stoichiometry.

3.2. Formation of a 2:1 GL020924–DNA complex is observed in ESI-MS

We employed ESI-MS to monitor the complex formation so that the binding stoichiometry could be determined unambiguously from the resulting mass spectrum. In order to validate

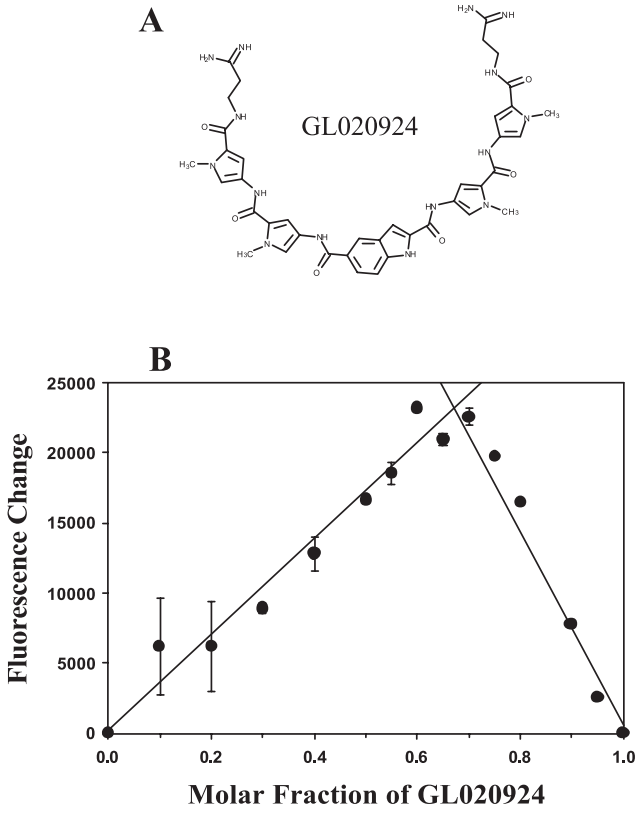

Fig. 1. A: The chemical structure of GL020924. B: Binding stoichiometry of GL020924 to FQ12 monitored by fluorescence changes in a continuous variation assay [17]. The total concentration of GL020924 and FQ12 was kept at 0.5 μM while the molar fraction of GL020924 was varied from 0 to 1. Rising and declining regions were fitted by linear regression.

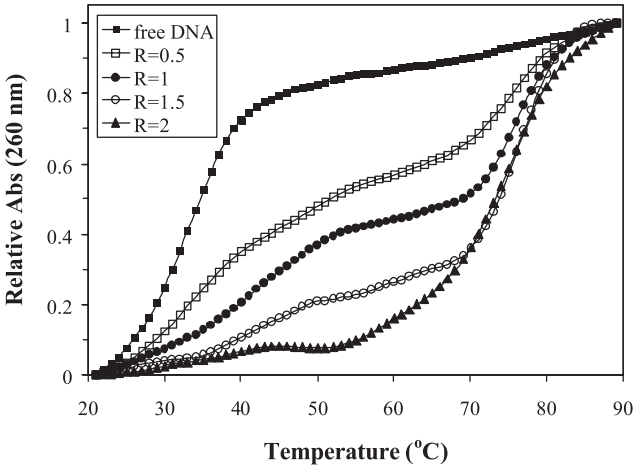

Fig. 2. DNA thermal melting monitored by UV absorbance at 260 nm. From left to right, the melting curves represent GL020924 to DNA ratios (R) of 0, 0.5, 1, 1.5 and 2, respectively.

that B-DNA conformation is not altered in the methanol-containing solvent used for MS analysis, we utilized CD spectroscopy to examine the duplex conformation prepared under different solvent conditions. The CD results showed similar characteristic B-DNA spectra for the duplex in HEN buffer, 15 mM ammonium acetate, and 15 mM ammonium acetate with methanol up to 30% (data not shown). This observation suggested that the presence of 22% methanol in the final sample for ESI desolvation should not alter B-DNA structure, consistent with previous observations [18]. Fig. 3 shows ESI-MS results of the binding of GL020924 to the 15 bp duplex with a central 8 bp A/T stretch. When the 15-mer duplex was mixed with GL020924 in a molar ratio of 1:0.9 (Fig. 3A), the major signals for the complex (ds+L) corresponding to 1:1 binding at charge states of 5, 6, and 7 were observed. A small amount of 2:1 complexes (ds+2L) with six charges also was observed. Additionally, we also detected a high abundance of single-stranded ODN ion signals, indicating substantial dissociation during the ESI process [19]. At $R = 2.2$, the most abundant peak in Fig. 3B corresponds to the six-charged 2:1 complex $(ds+2L)^{6-}$. The relative abundance of the 2:1 and 1:1 complexes is at the lower end of those of distamycin at similar

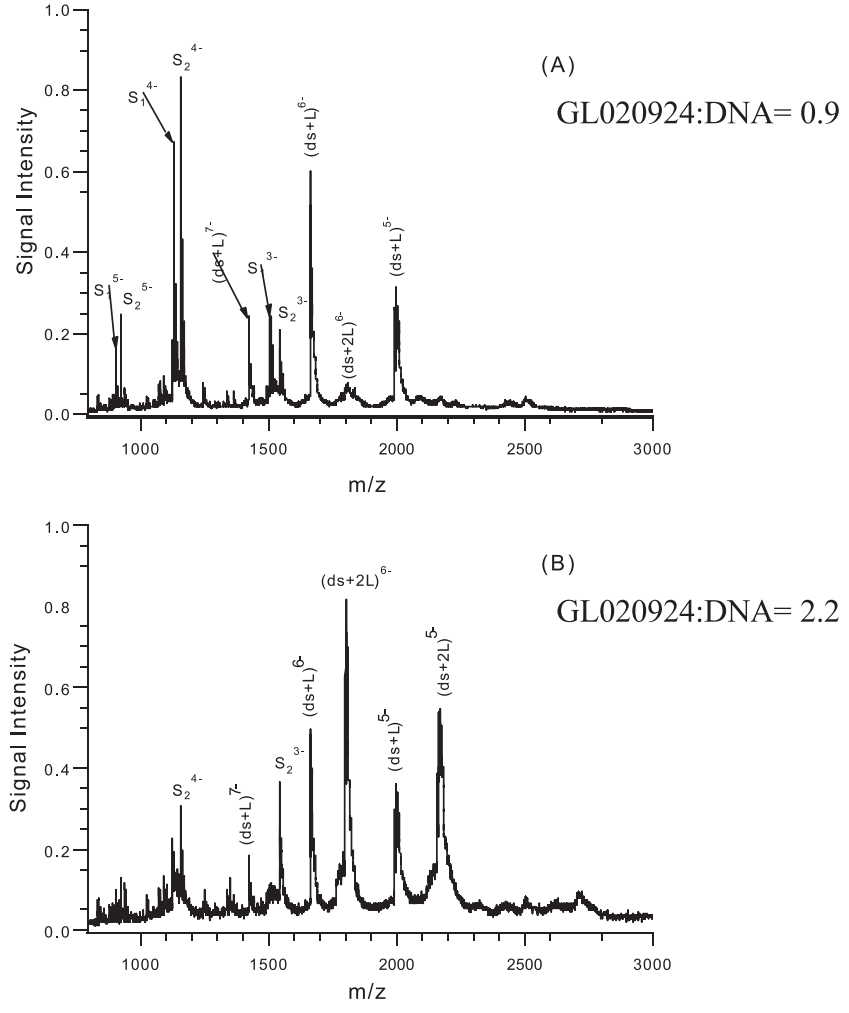

Fig. 3. Negative ion ESI mass spectra of a 15 bp DNA duplex mixed with GL020924 at the molar ratio of (A) 1:0.9; (B) 1:2.2. Peaks labeled S_1 and S_2 are the single-stranded 15-mer ODN (5′-dGCCAAATTTAACCGC-3′ molecular weight (MW) = 4521) and its complement (5′-dGCGGTTAAATTTGGC-3′ MW = 4623), respectively. In the figure, ds represents the duplex of S_1 and S_2, L for GL020924, ds+L for the 1:1 complex, and ds+2L for the 2:1 complex of GL020924 and the duplex.

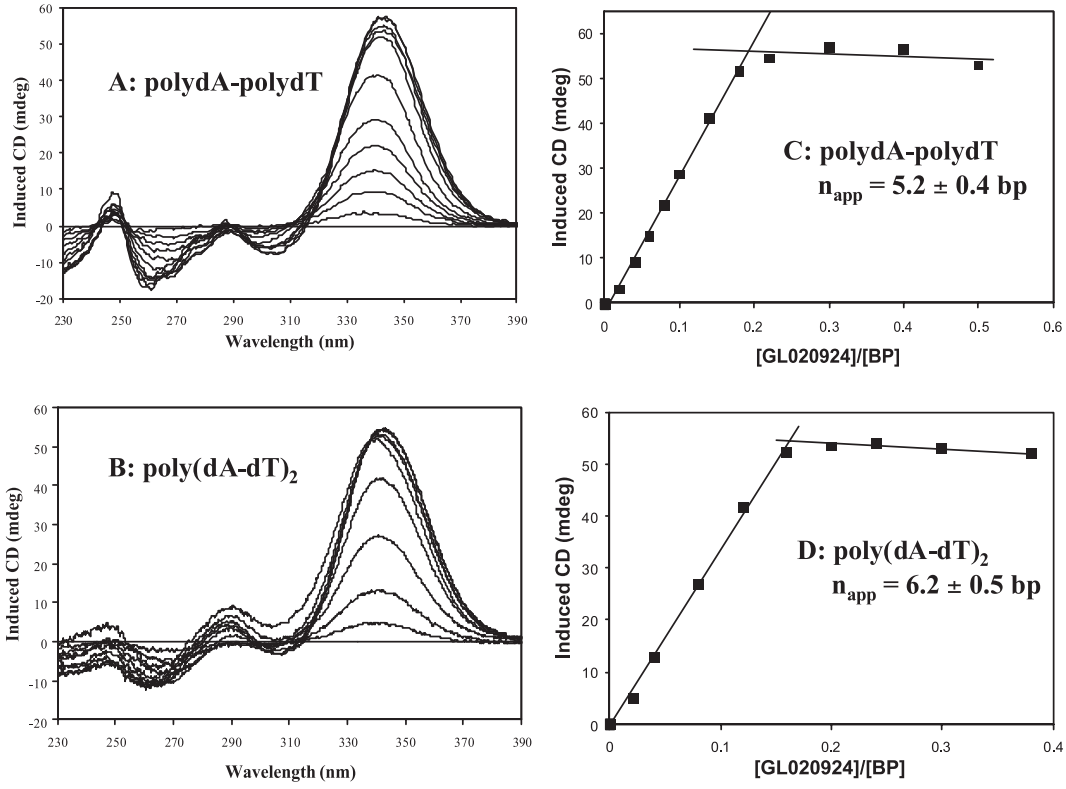

Fig. 4. CD spectrum for binding of GL020924 to (A) poly(dA)–poly(dT) and to (B) poly(dA–dT)$_2$. From bottom to top at 340 nm, the induced CD spectra represent ligand concentrations of 1, 2, 3, 4, 5, 7, 9, 11, 15, 20 and 25 μM in (A); and 1, 2, 4, 6, 8, 10, 12, 15 and 19 μM in (B). Analysis of GL020924-induced CD spectra at 340 nm for (C) poly(dA)–poly(dT) and (D) poly(dA–dT)$_2$. The intensity of the peak at 340 nm was plotted as a function of ligand to bp ratios.

ratios [18,20], indicating there may be some weak cooperativity in the binding of GL020924 to the 15-mer ODN.

3.3. *CD indicates GL020924 may form a staggered dimer upon binding to the DNA minor groove*

We used CD spectroscopy to determine the binding site size of GL020924 on polymeric poly(dA)–poly(dT) and poly(dA–dT)$_2$. Use of homogeneous polymeric DNA eliminates the end effects that are associated with short ODNs and the complication that mixed sequences may give rise to different binding modes and affinities. GL020924, itself, does not exhibit a CD spectrum, as it contains no chiral centers. The CD spectra were analyzed as differences between spectra of the DNA with varying amounts of ligand and the DNA alone. The strong positive peaks centered at 340 nm, as shown in Fig. 4A,B, are consistent with binding interactions in the minor groove. Since the induced CD signal is unique to the ligand–DNA complex, the peak values at 340 nm can be used to quantify the extent of complex formation. Induced CD signals at 340 nm were thus plotted against the ratio of ligand to DNA bp and analyzed through linear regression in Fig. 4C,D. The intercepts were determined to be 0.19 ± 0.01 for poly(dA)–poly(dT) and 0.16 ± 0.01 for poly(dA–dT)$_2$, corresponding to apparent binding site sizes (n_{app}) of 5–6 bp per GL020924 molecule. However, based on the molecular contour length and its rigid conformation, GL020924 should occupy approximately 8 bp in DNA minor groove. Therefore, the CD results are most consistent with formation of a partially overlapping dimer in the minor groove spanning 10–12 bp ($2 \times n_{app}$). This apparent site size is consistent with previous

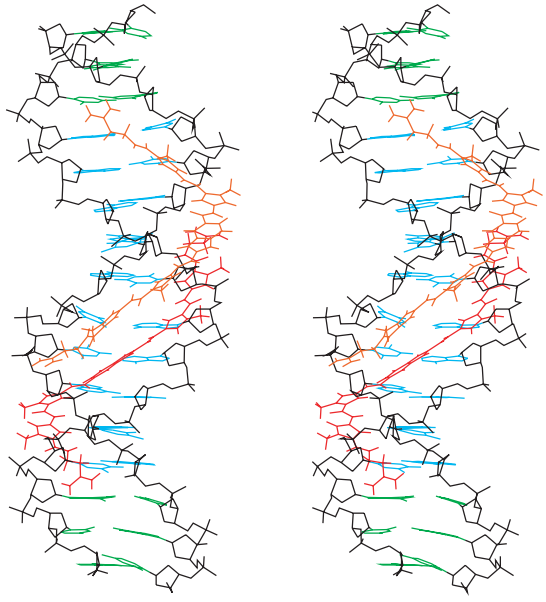

Fig. 5. Stereoview of the model of the minor groove complex between a 15 bp DNA and two GL020924 molecules. A/T bases are shown in blue, G/C bases are shown in green, backbone atoms are shown in black, and GL020924 molecules are shown in orange and red. Hydrogen atoms are omitted for clarity.

DNase I footprinting data [16,17]. It is likely that GL020924 forms a staggered dimer to minimize repulsion between charged amidinium ends.

3.4. Conformational changes may occur upon GL020924–DNA binding

A molecular model of the putative 2:1 GL020924–DNA complex (Fig. 5) was built as described in Section 2. In this model, where the GL020924 stack intentionally covers close to 12 bp, one amidinium tail of each ligand stacks on the penultimate pyrrole carboxamide unit of the other ligand, which could be a very efficient way to minimize charge repulsion. In accordance with experimental data, the model suggests that the two overlapped GL020924 molecules can dimerize within 8–10 bp A/T stretches with charge groups occupying extra 1–2 non-specific bp sequences at each end. The proposed GL020924 stacking mode must involve, to some extent, parallel stacking with respect to the amide bond, for which, hitherto, no experimental evidence has been reported. Our modeling studies also show clearly that GL020924 is too curved to penetrate the minor groove of canonical B-DNA deeply along its entire length, suggesting the DNA conformation may be altered upon ligand binding. In our model, the van der Waals interaction between the GL020924 stack and the DNA minor groove would improve if the A/T tract was curved slightly towards the major groove.

The unique structure and DNA binding behavior of GL020924 add to the diversity of known minor groove ligands. It has been shown previously that a benzimidazole-containing dicationic compound (without amide bond linkage) can form a dimer in the DNA minor groove [12,13]. Our results provide evidence of the first dicationic polyamide that is capable of dimerizing in the minor groove. The hydrophobic character of the indole moiety and the specific head-to-head orientation of GL020924 may explain why GL020924 binds DNA as a dimer. We have observed that dicationic analogs of GL020924 with smaller ring moieties can form only 1:1 complexes in the DNA minor groove (W.Z., unpublished data), indicating stacking interactions among smaller ring systems may not be sufficient to drive dimer formation. Based on molecular modeling (Fig. 5) and the apparent binding site size (Fig. 4), we propose that the ligand overlap must involve, to some extent, parallel amide bond stacking of GL020924. The peptide bonds give rise to sufficient flexibility to allow two GL020924 molecules to stack and still retain an isohelical conformation. Consistent with CD data, we believe that the dimer is only partially overlapped to minimize the charge repulsion between two amidinium groups. Structural studies of the 2:1 complex between GL020924 and DNA to determine the exact bound conformation are ongoing.

Finally, we wish to note that a family of GL020924 analogs have exhibited potent antifungal and antibacterial activities (data not shown) in addition to capabilities for gene regulation [16]. The novel DNA binding behavior likely accounts for the biological activities of GL020924 and structurally related compounds.

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References

[1] Bailly, C. (2000) Curr. Med. Chem. 7, 39–58.
[2] Boykin, D.W., Kumar, A., Spychala, J., Zhou, M., Lombardy, R.J., Wilson, W.D., Dykstra, C.C., Jones, S.K., Hall, J.E. and Tidwell, R.R. (1995) J. Med. Chem. 38, 912–916.
[3] Lown, J.W., Krowicki, K., Balzarini, J. and De Clercq, E. (1986) J. Med. Chem. 29, 1210–1214.
[4] Wemmer, D.E. (2000) Annu. Rev. Biophys. Biomol. Struct. 29, 439–461.
[5] Bostock-Smith, C.E., Harris, S.A., Laughton, C.A. and Searle, M.S. (2001) Nucleic Acids Res. 29, 693–702.
[6] Reinert, K.E. (1999) J. Biomol. Struct. Dyn. 17, 311–331.
[7] Jen-Jacobson, L., Engler, L.E. and Jacobson, L.A. (2000) Struct. Fold. Des. 8, 1015–1023.
[8] Spolar, R.S. and Record, M.T. (1994) Science 263, 777–784.
[9] Dickerson, R.E. (1998) Nucleic Acids Res. 26, 1906–1926.
[10] Storl, K., Burckhardt, G., Lown, J.W. and Zimmer, C. (1993) FEBS Lett. 334, 49–54.
[11] Pelton, J.G. and Wemmer, D.E. (1989) Proc. Natl. Acad. Sci. USA 86, 5723–5727.
[12] Wang, L., Bailly, C., Kumar, A., Ding, D., Bajic, M., Boykin, D.W. and Wilson, W.D. (2000) Proc. Natl. Acad. Sci. USA 97, 12–16.
[13] Wang, L., Carrasco, C., Kumar, A., Stephens, C.E., Bailly, C., Boykin, D.W. and Wilson, W.D. (2001) Biochemistry 40, 2511–2521.
[14] Lown, J.W., Krowicki, K., Balzarini, J., Newman, R.A. and De Clercq, E. (1989) J. Med. Chem. 32, 2368–2375.
[15] Fishleigh, R.V., Fox, K.R., Khalaf, A.I., Pitt, A.R., Scobie, M., Suckling, C.J., Urwin, J., Waigh, R.D. and Young, S.C. (2000) J. Med. Chem. 43, 3257–3266.
[16] Laurance, M.E., Starr, D.B., Michelotti, E.F., Cheung, E., Gonzalez, C., Tam, A.W., Deikman, J., Edwards, C.A. and Bardwell, A.J. (2001) Nucleic Acids Res. 29, 652–661.
[17] Kwok, K., Zhang, W., Schroth, G.P., Liang, C.H., Alexi, N. and Bruice, T.W. (2001) Biochemistry 40, 12628–12638.
[18] Gabelica, V., De Pauw, E. and Rosu, F. (1999) J. Mass Spectrom. 34, 1328–1337.
[19] Gale, D.C. and Sondhi, S.M. (1995) J. Am. Soc. Mass Spectrom. 6, 1154–1164.
[20] Triolo, A., Arcamone, F.M., Raffaelli, A. and Salvadori, P. (1997) J. Mass Spectrom. 32, 1186–1194.