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Aromatic Layered Guanidines Bind Sequence-Specifically to DNA Minor Groove with Precise Fit

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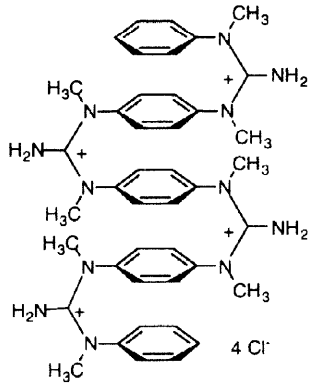
Abstract: Tetraguanidines (**1** and **2**) with an aromatic multilayered structure exhibit potent binding to a specific site of DNAs with binding constants of the order of 10^7 M^{-1} as deduced from Scatchard analysis of ultrafiltration data and simulation of CD titration curves. NMR spectra of the complexes indicated that **1** and **2** bind DNAs sequence-specifically at the minor groove, as predicted by computational docking studies.

Keywords: Guanidines; Nucleic acids; Molecular recognition; NMR

Compounds that bind to double-stranded DNAs, in particular, sequence-specific binders, may modulate gene replication or transcription.[1,2] Minor groove binders are a typical class of noncovalent DNA-binders.[3] Structurally, most of them consist of cationic functional groups such as amidino or guanidino groups at the terminal position(s) with a concatenated backbone of moderate planarity, as represented by netropsin, distamycin, and their synthetic analogs. The minor groove in typical B-DNA is narrow with a width of 5.7 Å and a depth of 7.5 Å,[4] but the pocket is large enough to accommodate compounds rather bulkier than conventional minor groove binders, as proved by the side-by-side dimeric arrangement of distamycin A in the groove,[5] and the sequence-specific recognition by polyamide dimers or their hairpin-type derivatives.[6] Based on the spatial size and the electronic properties of the minor groove, we selected the aromatic layered guanidines **1** and **2**, so that one molecule would fit precisely into the groove pocket.



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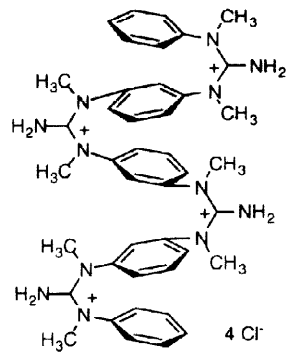

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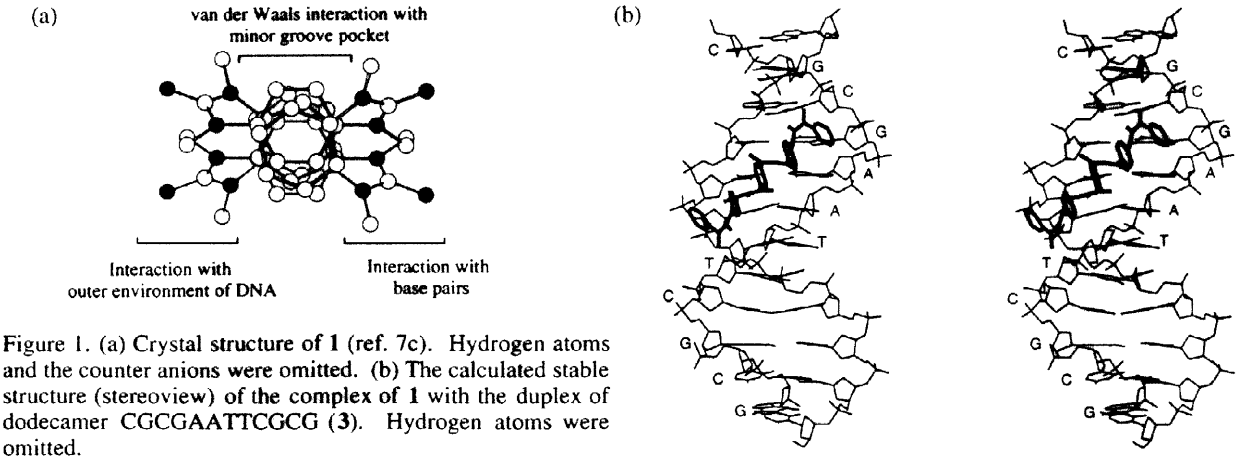

Figure 1. (a) Crystal structure of **1** (ref. 7c). Hydrogen atoms and the counter anions were omitted. (b) The calculated stable structure (stereoview) of the complex of **1** with the duplex of dodecamer CGCGAATTCGCG (**3**). Hydrogen atoms were omitted.

Cis conformational preference of *N,N'*-dimethyl-*N,N'*-diphenylguanidine made it possible to construct the hydrophilic aromatic multilayered compounds **1** and **2**.[7] In their structures, two cationic guanidino groups are located on both sides of the layered phenyl groups (Figure 1a), and we expected one side to interact with base pairs, and the other side to interact with the external environment (water, etc.) of DNAs. The aromatic layered skeletons were expected to undergo van der Waals interactions with the minor groove pocket. The validity of our approach was initially examined by a computational study using the automatic docking program ADAM.[8] The calculated stable structures of the complex of **1** and a DNA, the duplex of dodecamer CGCGAATTCGCG (**3**),[9] showed that **1** fits well into the minor groove of the duplex.[10] One of the most stable structures is shown in Figure 1b which is a complex at the 3'-(C)GAA(T)-5' region with one cationic side near the base pairs. The internalized guanidino groups may show hydrogen bonding to O(2) of T^8 and C^9 groups and N(3) of G^{10} group, as the distances are 3.0 – 3.1 Å. Compound **2** was also calculated to fit similarly to the minor groove, though **2** existed somewhat apart from the base pairs of **3** in the complex (data not shown).

The DNA-binding ability of **1** and **2** was evaluated by an ultrafiltration assay using calf thymus DNA (Table 1).[12] Both compounds bound strongly to DNA,[13,14] and the binding constants (*Ka*) of **1** and **2** calculated by Scatchard analysis were 6.2×10^6 and 1.2×10^7 M^{-1}, respectively, which are higher than that of

Table 1. Binding Abilities of Aromatic Layered Guanidines **1** and **2** with DNAs.

Compound	ultrafiltration assay [a]		ΔTm (deg) [b]		
	calf thymus DNA		calf thymus DNA	poly(dT)·poly(dA)	poly(dA-dT)$_2$
	Ka (M^{-1})	*n* (per base pair)			
1	6.2×10^6	0.20	10.3	29.8 [c]	25.1
2	1.2×10^7	0.19	9.9	25.8 [c]	22.2
netropsin	1.0×10^5	0.52	23.0	53.4	45.4

[a] Binding constant (*Ka*) and number of binding sites per base pair (*n*) were calculated from Scatchard analyses.

[b] ΔTm is defined as the difference of the melting temperature (*Tm* values) of DNAs in the presence and absence of the test compound. The molar ratio of the test compound to DNAs (per base pair) was 0.5. *Tm* values were measured at 260 nm, and those of calf thymus DNA, poly(dT)·poly(dA), and poly(dA-dT)$_2$ were 63.9, 45.4, and 39.0 °C, respectively.

[c] Biphasic *Tm* behavior was observed.

netropsin (1.0×10^5 M^{-1}) under the experimental conditions. The *Ka* values of the tetraguanidines were decreased concentration-dependently by the addition of NaCl. The number of binding sites (*n*) indicated that **1** and **2** occupy a five-base-pair length in the complexes. In contrast to the strong binding, the melting temperatures (*Tm*) for the DNA complexes of **1** and **2** were 74.2 and 73.8 °C, respectively, which are lower than that of the netropsin complex (Table 1). A similar result was observed when poly(dA)·poly(dT) or poly(dA-dT)$_2$ was used. When poly(dG-dC)$_2$ was mixed with **1** or **2**, only a mild, temperature-dependent hyperchromism was observed above 90 °C, and no distinct *Tm* was observed below 100 °C.

CD titration studies also indicated a potent interaction of the tetraguanidines with poly(dA-dT)$_2$. Interestingly, **1** and **2** exhibited different changes in CD spectra (Figure 2). The addition of **2** to a solution of poly(dA-dT)$_2$ increased both the negative and positive ellipticities at around 240 and 260 nm, respectively. On the other hand, apparent induced CD at 290 nm was observed in the case of **1**. In both cases, the increase of ellipticities was saturated at the tetraguanidine/DNA (per base pair) molar ratio of 0.5. The induced CD band disappeared when the temperature was raised, which suggests that the interaction of **1** or **2** with DNA is noncovalent. The *Ka* values, calculated by simulation of the CD titration data, were approximately 8×10^6 and 8×10^7 M^{-1} for **1** and **2**, respectively ($n = 0.17$ for both), which are somewhat larger than those obtained from the ultrafiltration assay. Furthermore, no significant change in the CD spectra was observed when **1** or **2** was added to poly(dG-dC)$_2$ solution at a molar ratio of tetraguanidines/DNA of below 0.5 (data not shown).

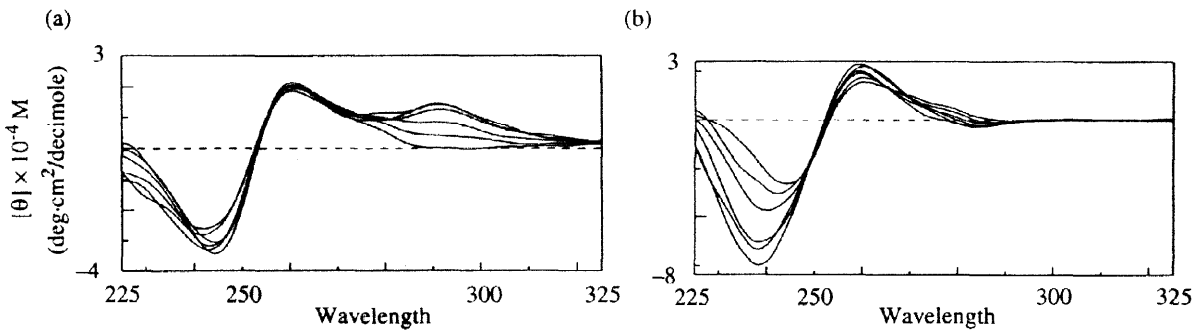

Figure 2. CD spectra of poly(dA-dT)$_2$ titrated with (a) **1** or (b) **2**. Concentration of DNA was 10 μM in TE buffer, and the ratio of the tetraguanidines to DNA was 0, 0.05, 0.1, 0.2, 0.5, or 1.

The binding structures of **1** and **2** were investigated by ^{1}H NMR spectroscopy using **3**.[14] The titration of a solution of the duplex **3** (1 mM) in 10 mM phosphate buffer (pH 7.0) with **1** caused shifts in the signals of the C(1') proton (sugar) at G^4, T^8, and C^9 to higher field (ca. 0.3 ppm on addition of 1 equiv of **1** to the duplex). Concerning the protons of base pairs, the signals of the C(2) protons of A^5 and A^6, and the C(5) proton of C^9 were shifted by addition of **1**. Similar changes were observed in the titration experiment using **2**, but to a lesser extent than in the case of **1**. The signals of the protons of **1** were also shifted ($\Delta\delta$: 0.2 – 0.5 ppm for the aromatic protons, and 0.2 ppm for methyl protons) in the presence of **3**, while the shifts were smaller in the case of **2**. The NOESY spectrum of the complex of **1** (or

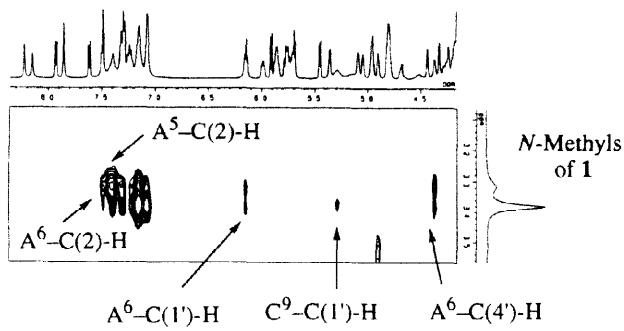

Figure 3. NOESY spectrum (300 msec) of a 1:1 mixture of **1** and the duplex **3** in 10 mM phosphate buffer (pH 7.0) at 35°C.

2) and 3 showed cross-peaks between the *N*-methyl protons of 1 and DNA sugar protons [C(1') and C(4') protons of A^6, and C(1') proton of C^9] or C(2) protons of the A^5 and A^6 residues (Figure 3). The NMR studies suggest that both 1 and 2 bind to the duplex 3 in the minor groove at around the 3'-GAA-5' region, in agreement with the result of the computational model study. This sequence-specificity may interpret the difference from netropsin with respect to ΔTm or CD titration.

In conclusion, we have designed novel DNA-binders with appropriate spatial shape and bulkiness to fit the DNA minor groove. *Cis*-guanidino groups act as building blocks for aromatic layers and also as hydrogen-bonding donors to base pairs. Further structural investigations should provide the basis for developing a new class of DNA groove binders.

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10. Two enantiomeric structures of 1 and 2 were prepared by inverting the coordinates of the crystal structure, and at first the conformations were kept rigid during the docking. Several tens of stable possible docking models obtained by ADAM (ref. 8) runs were further optimized by the AMBER program package (ref. 11), allowing the movement of both the DNA duplex and 1 (or 2). The stable docking model shown in Figure 1b was selected based on the sum of the interaction energy between DNA and 1 and the intramolecular energy of 1.
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13. Ultrafiltration assay was performed as follows: A mixture of a test compound and calf thymus DNA (0.1 mM) in TE buffer [10 mM Tris·HCl (pH 8) – 1 mM EDTA] was heated at 95 °C for 3 min, and allowed to stand at room temperature overnight. The mixture was ultrafiltered using a Microcon-10 UF tube (Amicon Co.) at 20 °C, then the concentration of the test compound in the filtrate was determined by HPLC (column Shiseido UG120Å). DNA-binding amount was calculated as the difference between the contents of the test compound in the filtrate in the presence and absence of DNA. The experiments using various concentrations of 1 or 2 afforded the binding constants (*Ka*) and number of binding sites per base pair (*n*) by Scatchard analysis.
14. DNAs used in these experiments were confirmed to exist in B form by CD spectroscopy.