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Strong Binding in the DNA Minor Groove by an Aromatic Diamidine with a Shape That Does Not Match the Curvature of the Groove

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A number of minor groove binders, including diamidine compounds (Figure 1), have been crystallized with the DNA duplex d(CGCGAATTCGCG)$_2$.[1] The compounds generally bind at the central AATT sequence and penetrate deeply into the minor groove such that the amidines can form H-bonds with the edges of the bases at the bottom of the groove.[2] Molecular curvature is critical for recognition, and compounds that have too great or too small a curvature cannot optimize contacts in the minor groove.[3] The importance of compound shape is seen with DB 75 and DB 359 (Figure 1), in which the amidine groups are in the para and meta positions on the phenyl ring.[4] The shape of meta analogue does not complement the curvature of the minor groove, and it binds significantly more weakly to AT DNA sequences and with lower specificity than the para compound, DB 75. Pentamidine analogues with four or six methylenes are more linear and bind to DNA significantly more weakly than pentamidine (Figure 1).[3]

Research on diamidines has demonstrated a correlation between the biological activity of these compounds and their structure and ability to bind to the AT minor groove.[5] Given this extensive and self-consistent data set, it is quite surprising that the meta-substituted diamidine, CGP 40215A (Figure 1), which has little curvature, has been found to have excellent antitrypanosomal activity.[6] The meta substitution and lack of curvature raise the question of whether CGP is the first biologically active diamidine that does not bind significantly to the DNA minor groove. This would bring into question the relevance of DNA binding to the biological activity of other minor groove binding compounds. On the other hand, if CGP 40215A does bind to the minor groove, its unusual shape raises other questions about what type of complexes it can form with DNA. To address these questions, we have used DNaseI footprinting, biosensor-surface plasmon resonance (SPR), X-ray crystallography, and molecular dynamics (MD) simulations to show that CGP 40215A binds strongly to the DNA minor groove.

DNase I footprinting is the method of choice for establishing the sequence specificities of DNA binding molecules. In a comparison study of different diamidines pentamidine, which is similar in length to CGP 40215A, gave weak footprints, whereas berenil and CGP 40215A strongly inhibited the cleavage of DNA by the enzyme at AT tracts (Supporting Information Figure S1). CGP 40215A gave stronger footprints than berenil but it is clear that, despite their significant differences in shape, the two compounds recognize common AT, but not GC, sequences.

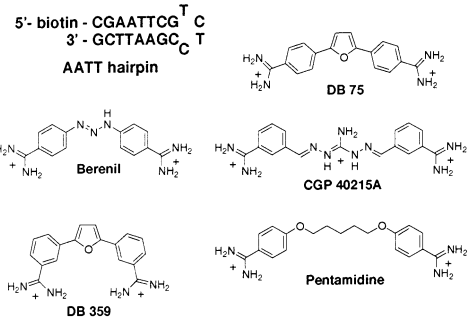

Figure 1. AATT DNA hairpin and diamidine compounds. CGP 40215A has three positive charges when bound to DNA.

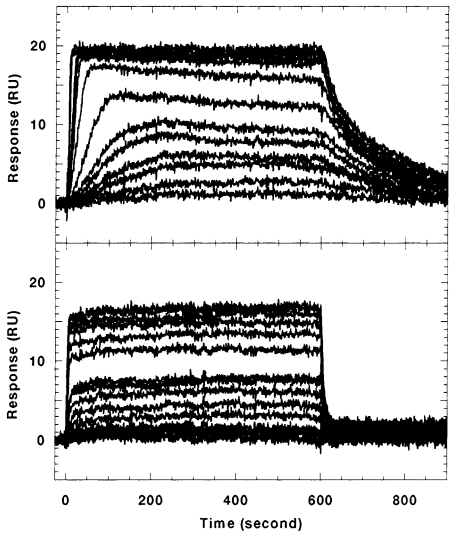

Figure 2. Typical sensorgrams for the interaction of CGP 40215A (top) and berenil (bottom) with the AATT DNA hairpin shown in Figure 1. The ligand concentrations increase from 1 to 650 nM from bottom to top.

The interactions of CGP 40215A and berenil with a specific AT DNA sequence were evaluated by using SPR experiments (Figure 2). The RU response increases with increasing compound concentrations as the compound binds to the DNA AATT sites. The maximum response at saturation (RU_{sat}) indicates that both compounds form 1:1 complexes with this DNA.[7] CGP 40215A exhibits slow on/off kinetics in contrast to the fast on/off rate for berenil at all concentrations (Figure 2). The averaged response units in the steady-state region were converted to r (RU/RU_{sat}) and plotted against the concentrations (Supporting Information Figure S2). Fitting of the results with standard methods shows that both CGP 40215A and berenil bind strongly to the AATT DNA. Strong

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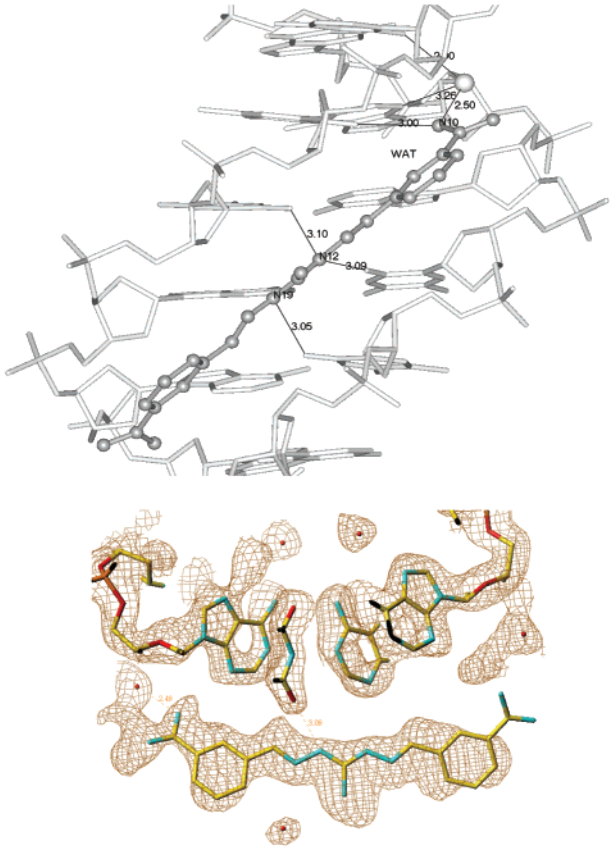

Figure 3. Structure of CGP 40215A binding to the minor groove of the AATT DNA sequence. The top figure is into the minor groove. H-bonds between the linker −NH groups and the amidine at the top of the figure and DNA bases are shown with solid black lines. The bottom figure is a side view of the electron density emphasizing the water-medicated interactions with the minor groove. Water molecules are indicated as red dots. (PDB 1M6F).

association was also seen with an alternating AT sequence, but very weak association, with an alternating GC sequence in agreement with the footprinting results. The binding strength of CGP 40215A ($K = 7 \times 10^7$ M^{-1}) is about six times higher than for berenil with the AATT hairpin (Figure 1).

CGP 40215A thus binds strongly as a monomer in the minor groove at AT sequences as with other biologically active diamidines. These experimental results, however, raise the very interesting and puzzling question of how the DNA minor groove is able to form strong interactions with the linear, meta-substituted compound. To address this question CGP was crystallized with the d(CGCGAATTCGCG)$_2$ duplex used to determine the structures of other diamidine−DNA complexes (Figure 3; crystallographic details are in Supporting Information). Diffraction data were collected to 1.78 Å, and the structure was solved by molecular replacement. The ligand was revealed as a continuous region of electron density in the minor groove (Figure 3). It fits deeply into the minor groove in the AATT sequence with the two −NH groups of the linker pointed into the groove to form direct H-bonds with the O2 groups of three thymines (Supporting Information Figure S3). Because of the linear structure of CGP, both amidines cannot form direct H-bonds with the bases. In the crystal structure one amidine forms an H-bond with the O2 of T8 and indirect, water-mediated H-bonds to the A that is complementary to T8 as well as to C9 (Supporting Information Figure S4). The amidine at the other end of the molecule is more dynamic, and it was modeled as being disordered, with 50% occupancy in each of two positions, pointing into and out of the minor groove.

To investigate these dynamic questions in more detail, MD simulations were conducted on the CGP 40215A complex with the DNA sequence used in the X-ray studies. The simulations were conducted as previously described[8] (details in Supporting Information). The simulations were started from several minor groove docking positions. In all cases, the molecule moved deeply into the groove at the AATT sequence and formed an array of dynamic H-bonds. A particularly favorable position was almost identical to that in the crystal structure with one amidine and the linker −NH groups forming direct H-bonds to DNA (Supporting Information Figure S5). The opposite amidine also points into the groove but has only water-mediated interactions with the bases. In the simulations, the two amidines were able to switch between direct and indirect base interactions. The linker serves as a "seesaw"-type hinge to allow strong interactions with the bases throughout the dynamic range of motions that switch the positions of the amidines to give two similar structures (Supporting Information Figure S6). The MD and crystal structures are thus in excellent agreement and show that CGP 40215A binds strongly to the minor groove in an ensemble of very similar structures with direct and water-mediated interactions. CGP (with its flexibility and linear structure and water-mediated DNA interactions) may require more time than berenil to optimize DNA interactions, and this could account for its slower association kinetics as observed in SPR. The complex has several particularly favorable structures that are linked through dynamic H-bonds, and this can account for its slower dissociation kinetics. The ensemble of linked structures results in high AT specificity and strong DNA binding for this unusually shaped minor-groove binding molecule. The CGP−DNA complex thus represents a new model for recognition and strong binding to the DNA minor groove in AT sequences. It will be useful to exploit this recognition motif to design new types of agents that can target DNA as part of their therapeutic action.

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Supporting Information Available: Figures S1−S6 and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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