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Structural Feature of Bent DNA Recognized by HMGB1

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Supporting Information

ABSTRACT: High Mobility Group Box 1 (HMGB1) protein, a potential therapeutic target, binds bent DNAs structure-specifically. Here we report on a crucial structural feature of the bent DNA required for strong binding to HMGB1. NMR structures of two bent DNA oligomers, only one of which binds strongly to HMGB1, revealed that the presence of a pocket structure on the minor groove is crucial for strong binding through penetration of a phenylalanine residue.

High Mobility Group Box 1 (HMGB1) protein is a highly abundant non-histone nuclear protein belonging to the High Mobility Group superfamily.[1] HMGB1 binds DNA in a structure- but not sequence-specific manner, where bent DNA structures such as cisplatin−DNA adducts (*CP−DNA*) are preferred over linear B-form DNA[1] (Figures S1 and S2 in the Supporting Information). HMGB1 has diverse functions related to its DNA binding ability. Within cells, HMGB1 regulates chromatin structure, V(D)J recombination, transcriptional regulation, and DNA repair.[2] Outside of cells, HMGB1 mediates inflammation processes.[3−5] HMGB1 is actively released from cells following cytokine stimulation and passively released during cell death. Released HMGB1 can activate the innate immune system.[3] HMGB1 is considered to be a potential therapeutic target because it mediates a wide range of physiological and pathological responses.[3,6] HMGB1 alone has little or no proinflammatory activity, but it appears to activate several immune receptors once complexed with antigenic nucleic acids.[4,5] The activation processes are inhibited by the presence of DNA that strongly binds to HMGB1.[4] Therefore, DNAs that have high affinity for HMGB1 may be candidates for use as anti-inflammatory agents. Although tertiary structures of *CP−DNA* and its complex with rat HMGB1 have been determined[7,8] (Figure S2), precise details concerning the types of bent DNA structures preferred by HMGB1 remain unknown.

Recently, novel DNA oligomers containing cyclic 2′-deoxyuridylate dimers that possess alkylene linkages between the 5-positions of uracil bases with various lengths have been developed.[9] Of these, DNA oligomers containing ethylene and propylene cross-linked cyclic 2′-deoxyuridylate dimers (called *Ethylene−DNA* and *Propylene−DNA*, respectively, in this manuscript) (Figure S3) were estimated to have deep bending angles (86 and 84°, respectively) on the basis of fluorescence resonance energy transfer (FRET) experiments.[9] However, their binding affinities for human HMGB1 A box as determined by gel-shift assays differed markedly: whereas *Ethylene−DNA* bound strongly to HMGB1 A box with a dissociation constant of ∼2 nM, *Propylene−DNA* did not exhibit strong binding.[9] These results were surprising because all bent DNAs were expected to bind HMGB1 strongly. In an effort to explain this affinity difference and gain new insight into the DNA-binding preferences of HMGB1, detailed structural analyses of these two DNA oligomers were performed.

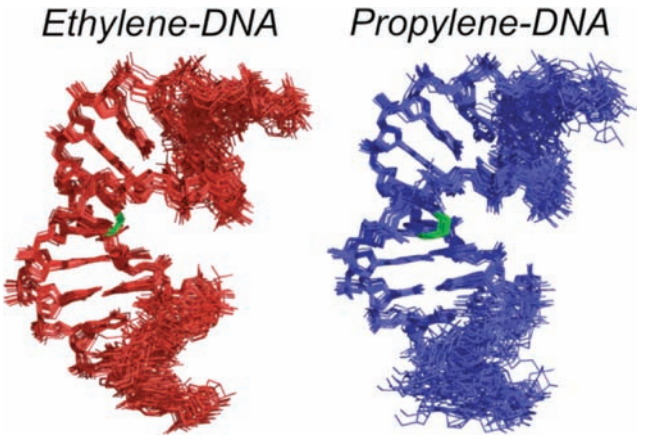

Figure 1. Superimposed representations of the 20 lowest-energy NMR structures of *Ethylene−DNA* (red) and *Propylene−DNA* (blue). The alkylene linkers are colored green.

High-resolution structures of the two 14-mer DNA oligomers *Ethylene−DNA* and *Propylene−DNA* were determined using solution NMR spectroscopy (Figure 1, PDB ID 2rrr and 2rrq, respectively). All of the ^{1}H resonances of these DNA oligomers except for H4′, H5′/H5″, and NH_2 of guanine were assigned.[10] From NOESY spectra in H_2O/D_2O, 309 and 307 ^{1}H−^{1}H distance restraints for *Ethylene−DNA* and *Propylene−DNA*, respectively, were obtained (Table S1 in the Supporting Information). All of the structural statistics and restraints are summarized in Table S2. In Figure 1, the red and blue structures represent superimposed pictures of the final 20 structures of *Ethylene−DNA* and *Propylene−DNA*, respectively. The overall structures of these two DNA oligomers are quite similar. Both DNA oligomers adopt overall right-handed B-form-like structures, although they have wider minor grooves than canonical B-form DNA (Figure S4) and sharp bends around the cross-linked site (green in Figure 1). The bending

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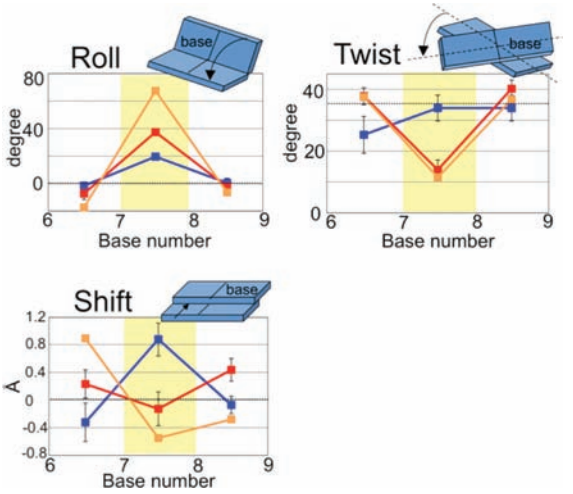

Figure 2. Selected helical parameters (roll, twist, and shift) calculated for all of the DNA oligomers around the cross-linked site. Red, *Ethylene−DNA*; blue, *Propylene−DNA*; orange, *CP−DNA* complexed with rat HMGB1 (PDB entry 1CKT).[8] Dotted lines show the value for canonical B-form DNA.

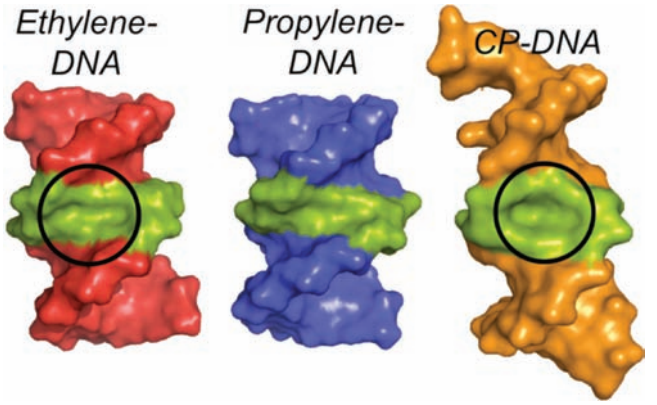

Figure 3. Surface representations of the lowest-energy structures (rotated 90° around the vertical axis in Figure 1) of (left) *Ethylene−DNA*, (middle) *Propylene−DNA*, and (right) the *CP−DNA* complex. Cross-linked bases and the complementary bases are shown in green. Pockets observed in the cross-linked sites are circled.

angles of *Ethylene−DNA* and *Propylene−DNA* were found to be 82 ± 5 and 79 ± 7°, respectively (Table S3). These bending angles are identical to those determined using FRET[9] within the error limits, indicating that the NMR structure is consistent with the FRET data.

In contrast to the overall structure, the local structure adopted around the cross-linked site provides the basis for distinguishing *Ethylene−DNA* from *Propylene−DNA*. This structural difference was experimentally displayed by the H2 signal of *Propylene−DNA* A21 residue, which showed a marked upfield shift (6.32 ppm at 303 K; Figure S5A). This is due to a ring-current shift, as A21 H2 is located immediately above the aromatic ring of A9 (Figure S5B). This upfield shift was not observed for the corresponding H2 signal of *Ethylene−DNA*. In order to characterize the DNA local structure quantitatively, helical parameters were calculated. The obtained helical parameters, which show significant differences between *Ethylene−DNA* and *Propylene−DNA* around the cross-linked sites, are displayed in Table S3 and Figure 2. *Ethylene−DNA* has a larger

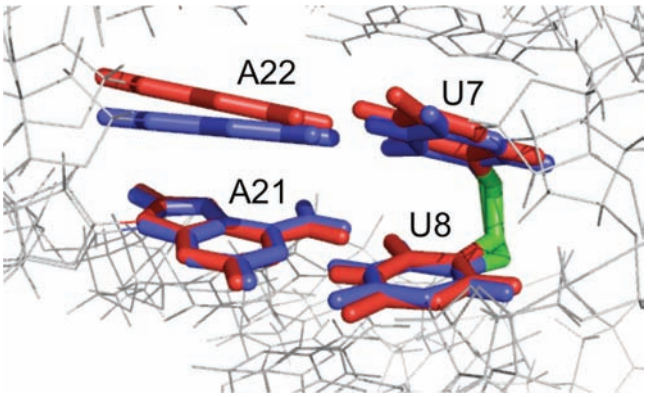

Figure 4. Expanded view of the lowest-energy structures of *Ethylene−DNA* (red) and *Propylene−DNA* (blue). Alkylene-linked bases and their complementary bases are shown as colored stick models. Alkylene linkages are shown in green.

positive roll angle and a smaller twist angle than *Propylene−DNA*. *Propylene−DNA* has a large positive shift value, although the value for *Ethylene−DNA* is almost zero. The differences in these helical parameters demonstrate the presence of a local structural difference between *Ethylene−DNA* and *Propylene−DNA* and provide insight into the bending mechanism. In *Ethylene−DNA*, the significantly large, positive roll angle combined with unwinding plays a major role in formation of the bent structure, while in *Propylene−DNA*, the significantly large, positive shift value and the positive roll angle seem important. Although the combination of a large, positive roll angle with unwinding for bending as found in *Ethylene−DNA* is quite common among bent DNAs,[11] to the best of our knowledge, this is the first report of a large positive shift value combined with a positive roll angle as found in *Propylene−DNA*.

The most significant structural feature of *Ethylene−DNA* is the presence of a pocket structure on the minor groove at the cross-linked site. Figure 3 shows surface representations of the minor-groove views of *Ethylene−DNA* and *Propylene−DNA*, with the pocket structure highlighted by a black circle. This pocket structure results primarily from the large, positive roll angle and the unwinding (Figures 2 and 4). The presence of the pocket structure is consistent with the fact that *Ethylene−DNA* is thermally less stable than *Propylene−DNA* (Figures S6 and S7). This pocket structure is also found at the cross-linked site in *CP−DNA* complexed with rat HMGB1[8] (Figure 3 and Figures S2 and S8), where the cross-linked bases are larger guanines instead of the smaller uracils used for *Ethylene−DNA* and *Propylene−DNA*. The helical parameters of *CP−DNA* around the cross-linked site were more similar to those of *Ethylene−DNA* than those of *Propylene−DNA* (Table S3, Figure 2, and Figure S9). *Ethylene−DNA* and *CP−DNA*, each of which possesses both the pocket structure and a high affinity for HMGB1, can be distinguished from *Propylene−DNA*, which possesses neither the pocket structure nor a high affinity for HMGB1. In the structure of the *CP−DNA* HMGB1 complex, the aromatic ring of Phe-37 of HMGB1 (Phe-38 in human HMGB1) penetrates into the pocket structure (Figure S2), and this phenylalanine penetration is important for strong binding.[8] Therefore, we assume that the presence of the pocket structure in free DNA is one key factor that contributes to the strong binding between bent DNA and HMGB1 and that *Ethylene−DNA* is recognized by HMGB1 in a manner similar to its interaction with *CP−DNA*.

In an effort to confirm this hypothesis, a point mutant of human HMGB1 A box (F38A) wherein the penetrating phenylalanine was

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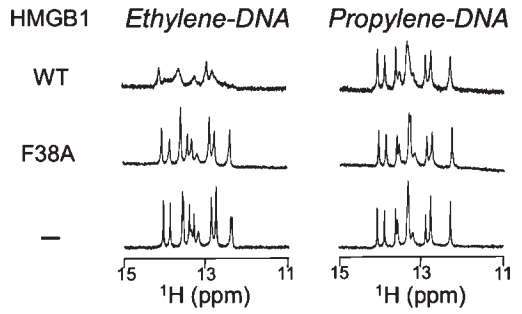

Figure 5. Imino proton regions of the 1D NMR spectra of (left) *Ethylene−DNA* and (right) *Propylene−DNA* without protein (bottom), with a 2-fold amount of HMGB1 (F38A) (middle), and with a 2-fold amount of HMGB1 (WT) (top).

replaced with alanine was prepared, and the importance of the pocket structure was evaluated by comparing the binding of the mutant with *Ethylene−DNA* and *Propylene−DNA*. Figure 5 shows the imino proton regions of the 1D NMR spectra of *Ethylene−DNA* and *Propylene−DNA* with excess amounts of wild-type HMGB1 A box (WT) and the F38A mutant. The imino proton signals of *Ethylene−DNA* exhibited marked line broadenings in the presence of WT but not with F38A. Neither WT nor F38A showed significant changes with *Propylene−DNA*. These results indicate that *Ethylene−DNA* binds HMGB1 strongly using the penetrating phenylalanine and thus support our hypothesis that the pocket structure of bent DNA is important for strong binding with HMGB1.

The DNA binding mechanisms of HMGB1 A box with *Ethylene−DNA* and *CP−DNA* were then compared. For *Ethylene−DNA*, the sharp signals that remained in the presence of WT were assigned to the T3, T12, G16, G27, and G28 bases, and the broadened signals were assigned to eight other central base pairs, including the cross-linked base pairs and three neighboring base pairs on each side. Therefore, WT directly binds both the 5′ and 3′ sides of the cross-linked base pairs of *Ethylene−DNA*. It is well-known that WT directly binds five base pairs in *CP−DNA*, including the cross-linked base pairs and the 3′ side of three neighboring base pairs (Figure S2).[8] It is conceivable that WT directly binds only one side of the cross-linked base pairs, for example, the 3′ side of *CP−DNA*, and therefore it is reasonable to assume that WT binds either side of *Ethylene−DNA* at any one time. The significant line broadening may be explained by exchanges between the free state and the two distinguishable complex states. With respect to binding direction, the DNA binding mechanism of *Ethylene−DNA* clearly differs from that of *CP−DNA*.

The present study has provided strong evidence to suggest that the bent structure of DNA alone is insufficient to account for the strong binding to HMGB1, although it has long been thought that HMGB1 binds all bent DNAs strongly. The bent structure of *Propylene−DNA*, which is not recognized by HMGB1, was determined and found to be unique because the bent conformation is induced by the large, positive shift value combined with the positive roll angle. This structure is thermally stable and may not permit deformation like that associated with residue penetration. The most important finding of this report relates to the presence of the pocket structure located at the bending site, and this is one of the key factors responsible for the strong binding as revealed by mutation studies. The pocket structure is likely to be important at the beginning of the interaction involving the penetrating phenylalanine of HMGB1, which results in expansion of the pocket structure. The low thermal stability of *Ethylene−DNA* is more favorable for residue penetration and stable complex formation, as rearrangements of the neighboring base positions are required for the penetration and stacking interactions. It is noted that the presence of the pocket structure seems to be more important than the low thermal stability because the thermal stability of *CP−DNA* is similar to that of *Propylene−DNA*.[9]

■ ASSOCIATED CONTENT

Supporting Information. Complete refs 4 and 5, materials and methods, structural restraints, and additional figures and results. This material is available free of charge via the Internet at http://pubs.acs.org.

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