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Sequence-Specific B-DNA Flexibility Modulates Z-DNA Formation

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Supporting Information

ABSTRACT: Conversion of right-handed B-DNA into left-handed Z-DNA is one of the largest structural transitions in biology that plays fundamental roles in gene expression and regulation. Z-DNA segments must form within genomes surrounded by a sea of B-DNA and require creation of energetically costly B/Z junctions. Here, we show using a combination of natural abundance NMR $R_{1\rho}$ carbon relaxation measurements and CD spectroscopy that sequence-specific B-DNA flexibility modulates the thermodynamic propensity to form Z-DNA and the location of B/Z junctions. We observe sequence-specific flexibility in B-DNA spanning fast (ps−ns) and slow (μs−ms) time scales localized at the site of B/Z junction formation. Further, our studies show that CG-repeats play an active role tuning this intrinsic B-DNA flexibility. Taken together, our results suggest that sequence-specific B-DNA flexibility may provide a mechanism for defining the length and location of Z-DNA in genomes.

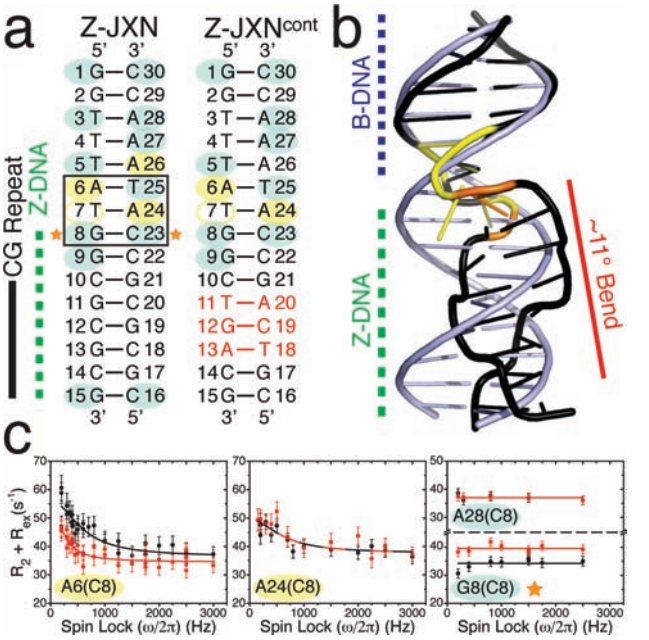

Figure 1. NMR observation of sequence-specific B-DNA flexibility in and around B/Z junctions. (a) B/Z junction forming DNA (Z-JXN) and control with disrupted CG-repeat (Z-JXN^{cont}) used in NMR studies. Differences between the two constructs are highlighted in red. Site of B/Z junction formation is highlighted with a box. Residues with detectable carbon chemical exchange in sugar (C1′) and base (C8) moieties are highlighted in open and filled yellow circles, respectively. Residues where chemical exchange was not detected are highlighted in blue. Residues with elevated ps-ns motions are highlighted with a star. (b) X-ray structure of the protein bound B/Z junction (black) aligned with a canonical B-form helix (gray). Residues with fast and slow motions when in the B-form state are color coded orange and yellow, respectively. (c) Representative on-resonance relaxation dispersion profiles measured for Z-JXN (black) and Z-JXN^{cont} (red).

One of the most dramatic transitions in biology is conversion of right-handed B-DNA into left-handed Z-DNA in sequences that are rich in pyrimidine/purine repeats. While the existence of Z-DNA *in vivo* was originally questioned, and indeed the subject of controversy, overwhelming experimental data accumulated over the past two decades unequivocally support its existence and role in fundamental processes such as transcription and nucleosome positioning.[1−3] In genomes, Z-DNA segments must form dynamically at specific locations with particular lengths and within a sea of B-DNA. The resulting formation of B/Z junctions is accompanied by energetically unfavorable conformational strain due to intersection of the drastically different B-DNA and Z-DNA structures.[4] While the sequence-dependence of Z-DNA formation has been studied extensively and shown to be dependent on both the length of pyrimidine/purine repeats and sequence of neighboring base-pairs,[5−7] these studies relied on bulk measurements that do not provide atomic level information about the length of Z-DNA or location of B/Z junctions. Indeed, the one and only X-ray structure of a B/Z junction[4] shows that Z-DNA can extend outside of CG-repeats to include other DNA steps, which based on accepted thermodynamic models[8] of Z-DNA formation is predicted to be highly energetically unfavorable. In addition, current methods used to predict the formation and location of Z-DNA in genomes do not yet account for the sequence specific energetic costs of creating B/Z junctions.[9] Thus, the rules that govern the length of Z-DNA segments and location of B/Z junctions remain poorly understood. Using natural abundance NMR $R_{1\rho}$ carbon relaxation measurements[10] in concert with CD spectroscopy, we show that sequence-specific B-DNA flexibility modulates the thermodynamic propensity to form Z-DNA. Our results add to a growing view that DNA sequences code for complex functionally important dynamics[11−13] spanning multiple time scales and also suggest that sequence-specific dynamics extends to long sequence repeats.

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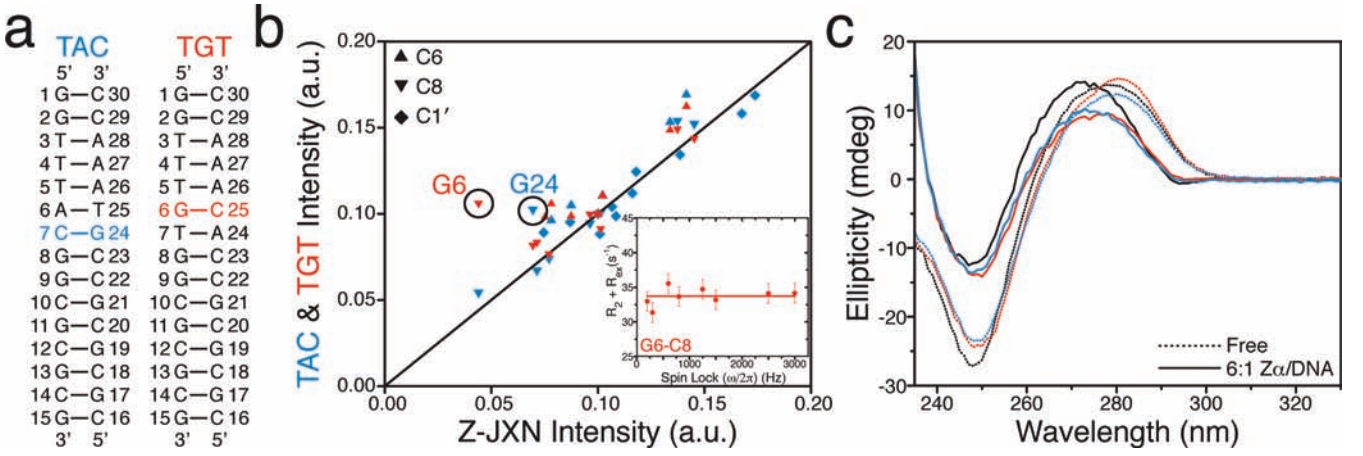

Figure 2. Sequence-specific preferences for Z-DNA formation. (a) Mutant constructs of Z-JXN with mutations highlighted in color. (b) Comparison of normalized $^{1}H-^{13}C$ HSQC peak intensities reveal differences in dynamics between constructs studied by NMR.[14] An A-to-G mutation at A24 (TAC) or A6 (TGT) eliminates exchange broadening and $\mu s-ms$ motions as confirmed for G6(C8) by relaxation dispersion (inset). Relaxation dispersion measurements could not be carried out on G24(C8) due to spectral overlap. (c) CD spectra of DNA free and bound to Zα protein color coded according to construct: black, Z-JXN; red, TGT; blue, TAC. CD samples were all the same DNA concentration.

The X-ray structure of a B/Z junction was reported for the sequence 5′ GGTTTATGGCGCGCG 3′ (Z-JXN) bound to the Z-DNA binding protein domain, Zα, of the RNA adenosine deaminase (ADAR1) protein.[4] Surprisingly, the left-handed helix extended beyond the boundary of the $(CG)_3$ repeat to include a CC step (Figure 1a), which according to a widely accepted Z-DNA thermodynamic model is energetically unfavorable due to the enhanced energetic cost accompanying the *anti*-to-*syn* transition of pyrimidines.[8] This localizes the B/Z junction at the T7-A24 base-pair, which is extruded from the double helix, allowing continuous stacking between B-DNA and Z-DNA (Figure 1a,b).

To obtain insights into the unusual behavior of Z-JXN upon undergoing the B/Z transition, we used natural abundance NMR $R_{1\rho}$ carbon relaxation dispersion experiments[15−17] to characterize its intrinsic dynamic properties in the B-form state, with no Z-DNA present, over broad (ps−ns and $\mu s-ms$) time scales and compared these findings to a control construct ($Z\text{-}JXN^{cont}$) in which the CG repeat was disrupted (Figure 1a). The $R_{1\rho}$ NMR experiments revealed chemical exchange involving slow ($\mu s-ms$) conformational transitions that likely involve the disruption of stabilizing interactions in B-DNA, such as hydrogen bonding and/or stacking interactions for residues in and around the site of B/Z junction formation (Figure 1a,b). Chemical exchange was observed for both the thymine and adenine residues in the T7-A24 base-pair, which becomes extruded upon B/Z junction formation.[4] For T7, the exchange is localized in the sugar (C1′) whereas for A24 it is localized in the base (C8). Nowhere else do we observe exchange in both Watson−Crick partners. Thus, the T7-A24 base-pair, which is significantly distorted following the B-to-Z transition, is already dynamic and flexible in the B-state. We also observe chemical exchange in the base moieties of neighboring adenines A6(C8) and A26(C8) but not in their corresponding thymine partner. All of the above residues are part of CA or TA pyrimidine/purine dinucleotide steps, which are known to be the dinucleotide steps with the weakest stacking interactions.[18−21] Thus, the observed exchange likely arises from instability in canonical B-DNA which results in enhanced propensities to access alternative conformations at these sites. The unique exchange observed for T7(C1′) is likely encoded by the TAT sequence and may reflect interhelical bending observed in the context of the extensively studied "TATA" box.[22] Indeed, the TAT site also acts as a hinge for helical bending in the B/Z junction X-ray structure (Figure 1b and S1). No exchange was observed for all other residues examined, including A27, A28, and T3 (Figure S2). However, this does not rule out the presence of conformational dynamics that falls outside the detection limits of our experiments.

To examine if the CG repeats play a role in the motions observed, we performed natural abundance carbon relaxation dispersion experiments on a control construct, $Z\text{-}JXN^{cont}$, in which the CG repeat was disrupted (Figure 1a). Chemical exchange was detected in the same residues of $Z\text{-}JXN^{cont}$ which suggests that we observe intrinsic B-DNA flexibility, rather than a unique deformation only accessible to samples able to undergo the B/Z transition. While disruption of the CG repeat did not affect the exchange observed at T7-A24 (Figure 1c and Figure S2), it significantly diminished the chemical exchange observed at A6(C8) which is more than three base-pairs away from the CG-repeat (Figure 1c). It is possible that this long-range effect propagates via the backbone without leading to observable effects on T7-A24, or perhaps because these fall outside the detection limits of the NMR experiment. Disruption of the CG repeat also significantly increased the intrinsic carbon transverse relaxation rate constants ($R_{2,int}$) and therefore decreased the apparent ps−ns motions at G8-C23, which is one of the two base-pairs in the CC step that unexpectedly form Z-DNA despite being outside the CG-repeat (Figures 1c and S3). This, along with unique chemical shift perturbations induced by CG-repeats (Figure S4), strongly suggest that CG-repeats modulate the dynamic properties of its neighboring residues.

Our results suggest that Z-DNA unexpectedly incorporates the C22, C23 step in Z-JXN in part because this makes it possible to localize the B/Z junction at the intrinsically flexible region centered at the T7-A24 site. To test this hypothesis, we measured the propensity to form Z-DNA upon binding to the Z-DNA binding domain, Zα, of ADAR1 in DNA constructs that bear point mutations within and above the junction designed to disrupt the inherent flexibility observed by NMR without disrupting interactions with the Zα protein which exclusively interacts with Z-DNA elements below the B/Z junction.[4] We

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assessed the propensity to form Z-DNA by recording circular dichroism (CD) spectra following incubation of each DNA construct with Zα protein at a 6:1 DNA:protein ratio and quantifying the extent to which the B-DNA spectrum is inverted as a result of Z-DNA formation.[23]

Replacement of the exchange broadened T7-A24 base-pair with a corresponding C7-G24 base-pair that shows no sign of NMR exchange broadening (Figure 2b) resulted in a significantly reduced (~15−20%) propensity to form Z-DNA (TAC, Figure 2c). Similar results were obtained when replacing the nearby exchange broadened A6-T25 base-pair with a G-C base-pair (TGT, Figure 2b,c). Further, in both the TAC and TGT constructs, the exchange broadening at T7(C1′) was diminished, indicating that flexibility at this site is an important factor for Z-DNA formation. Both mutants show similar extents of Z-DNA formation consistent with a change in the location of the B/Z junction in which Z-DNA retreats to the expected position at the end of the CG repeat, away from the newly introduced "rigid" residues. The same trends were observed when inducing the B-to-Z transition using conditions of high salt without the Zα protein (Figure S6), again confirming that the different propensities observed reflect intrinsic properties of the DNA. Taken together, these data indicate that sequence-specific flexibility in Z-JXN directs Z-DNA to form outside the CG repeat so to position the highly flexible A6-T25 and T7-A24 base-pairs at the junction.

In conclusion, our results show that CG-repeats affect the dynamic properties of neighboring residues and that this together with inherent sequence-specific B-DNA flexibility modulates the thermodynamic propensity to form Z-DNA. Sequence-specific B-DNA flexibility may provide a mechanism for controlling the length and location of Z-DNA in genomes.

Recently, while this manuscript was in submission, a study utilizing single-molecule fluorescence reported that intrinsic dynamics guides the B-to-Z transition in methylated DNA samples containing Ni^{2+}.[24] These results are consistent with our findings showing that intrinsic DNA dynamics plays an important role in the B-to-Z transition.

■ ASSOCIATED CONTENT

Supporting Information. Details of sample preparation, spectroscopic methods, structure comparison, dispersion profiles used for comparison, chemical shift analysis, high salt CD studies, and NMR assignments of key residues. This material is available free of charge via the Internet at http://pubs.acs.org.

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