The structures of non-CG-repeat Z-DNAs co-crystallized with the Z-DNA-binding domain, hZαADAR1

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ABSTRACT

The Z-DNA conformation preferentially occurs at alternating purine-pyrimidine repeats, and is specifically recognized by Zα domains identified in several Z-DNA-binding proteins. The binding of Zα to foreign or chromosomal DNA in various sequence contexts is known to influence various biological functions, including the DNA-mediated innate immune response and transcriptional modulation of gene expression. For these reasons, understanding its binding mode and the conformational diversity of Zα bound Z-DNAs is of considerable importance. However, structural studies of Zα bound Z-DNA have been mostly limited to standard CG-repeat DNAs. Here, we have solved the crystal structures of three representative non-CG repeat DNAs, d(CACGTG)2, d(CGTACG)2 and d(CGGCCG)2 complexed to hZαADAR1 and compared those structures with that of hZαADAR1/d(CGCGCG)2 and the Zα-free Z-DNAs. hZαADAR1 bound to each of the three Z-DNAs showed a well conserved binding mode with very limited structural deviation irrespective of the DNA sequence, although varying numbers of residues were in contact with Z-DNA. Z-DNAs display less structural alterations in the Zα-bound state than in their free form, thereby suggesting that conformational diversities of Z-DNAs are restrained by the binding pocket of Zα. These data suggest that Z-DNAs are recognized by Zα through common conformational features regardless of the sequence and structural alterations.

INTRODUCTION

DNA can adopt various secondary conformations other than the classical right-handed B-DNA structure under certain specific physiological conditions (1). Left-handed Z-DNA has been studied in detail by numerous methods over the last two decades since its first crystal structure was solved (2). Z-DNA conformations in many different sequences have been a challenge to crystallize. Nonetheless, several structures have been determined by X-ray crystallographic study (3). Many characteristic features of the Z-DNA structure have been identified from the accumulated structural data. The overall shapes of most Z-DNA structures share common features that are similar to those seen in the first crystallized Z-DNA structure of d(CGCGCG)2 (2). Z-DNA has a zig-zag sugar phosphate backbone and is longer and thinner than B-DNA. Its nucleotides form in a dinucleotide repeat in which they alternate with syn and anti conformations. Z-DNA is favored in alternating pyrimidine-purine (APP) sequences (4). The alternating CG-repeat sequence is the most favorable energetically for Z-DNA formation (5). Networks of water molecules hydrate Z-DNA, forming hydrogen bonds with atoms in both the backbone and base. However, some Z-DNAs form with different structural features, especially those with sequences without APP or including A-T base pairs (6–8). When A-T base pairs are introduced, the overall Z-DNA structure

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Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.rcsb.org (PDB ID codes of hZαADAR1 in complex with d(CACGTG)2, d(CGTACG)2 and d(CGGCCG)2 are 3F21, 3F22 and 3F23, respectively)

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becomes partially distorted due to disruption of the hydration spine (6). In addition, the non-APP base pairs have been found to be highly buckled when compared with other base pairs in Z-DNA (7,8). Up to now, however, there have been only a limited number of structural studies of Z-DNAs containing non-APP or A-T base pairs. Most studies have been carried out in the presence of excess cations or using dsDNAs modified by methylation or bromination. Thus, study of Z-DNA under low salt conditions or in the presence of Z-DNA-binding domains (Zx) remains to be explored, and this may provide insight into the effect that sequence variability has on Z-DNA conformation under physiological conditions.

The protein Zx domain was first identified from human ADAR1 (double-stranded RNA adenosine deaminase) and subsequently found in other proteins (DLM1, E3L and PKZ). These domains provide a unique opportunity to explore Z-DNA and its various roles in biological systems (9–13). The Zx domains are highly specific for the Z-conformation of nucleic acids, including dsDNA, dsRNA as well as DNA–RNA hybrids. They have binding affinities in the low-nanomolar range. In the crystal structures of double-stranded d(CGCGCG)2 complexed with hZxADAR1, mZxDLMI, ybZxE3L or hZxPKZ (10,11,14–16), a close resemblance was found between the bound and unbound states of the Z-DNA structure of d(CGCGCG)2 (2). It is now known that Z-DNA is a transcriptional promoter in the genome as an active transcription modifier that functions by modulating chromatin structure (17,18). The Z-DNA conformation is not limited only to APP sequences, but can appear in many other nucleotide sequences. Recent functional studies of the Z-DNA-binding domains in vaccinia E3L showed that it acts as a transcription modulator of several apoptosis-related genes (19). More recently, the Z-DNA-binding protein DLM1 has been found to act in the innate immune system as a cytosolic receptor for dsDNA, recognizing foreign pathogenic DNA (20). These results support the idea that diverse sequences of Z-DNA can be recognized by Z-DNA-binding domains and that their binding is essential for cellular processes. To understand the binding mode of Zx to Z-DNAs in various sequence contexts, it is necessary to investigate structural features of Z-DNAs bound to Zx and to compare their structures with Z-DNAs stabilized by base-modification and/or positively charged ions. In this regard, we undertook a study to solve Z-DNA structures with non-CG-repeat sequences stabilized by the same Zx domain. Here we report the co-crystal structures of three non-CG-repeat Z-DNAs containing either A-T base pairs or non-APP sequence bound to hZxADAR1. These complexes reveal how the structural diversity of Z-DNA caused by non-CG-repeat sequences is recognized and stabilized by the Z-DNA-binding domain.

**MATERIALS AND METHODS**

**Expression and purification**

hZxADAR1 (residues 133–209) from human ADAR1 was expressed and purified as described previously (21).

In brief, hZxADAR1 was purified through sequential chromatographic steps involving HiTrap metal affinity column (GE Healthcare, Piscataway, NJ), thrombin digestion for the removal of N-terminal his-tag and a Resource S column (GE Healthcare, Piscataway, NJ). The purity and concentration of hZxADAR1 were estimated by SDS-PAGE and the Bradford method, respectively. DNAs were purchased (IDT, Coralville, IA) and purified as described previously (22).

**Crystallization**

The DNA used for crystallization all had 6nt, plus a 5’ overhang which acts as a stabilizing capping residue. For crystallization, hZxADAR1 was mixed with dsDNA [(dT(CGGGCG)), (dTCGACG)], (dT(CGGCGG)G) or (dTCGGCGG)] at a 0.66 mM : 0.33 mM molar ratio in 5 mM HEPES-NaOH pH 7.5 containing 20 mM NaCl, and incubated at 303 K for at least 2 h. All crystallization experiments were performed using the hanging drop vapor diffusion method with a VDX plate at 295 K. Among the four dsDNA used in crystallization, only (dT(CGGCGG)) was successfully co-crystallized with hZxADAR1 when ammonium sulfate was used as precipitant. For crystallization of the other three complexes, crystals of hZxADAR1/d(TCGGCCG) were used as seeds, and initial crystals were again used as seeds for further crystallization. Diffraction quality crystals were obtained within a month using 2.2 M ammonium sulfate and 10% glycerol in the reservoir solution.

**Data collection and structure determination**

Preliminary X-ray diffraction analyses were performed at beamline BL6A of PAL (Pohang, Korea). X-ray diffraction data of frozen crystals were collected at 100 K with a MAR CCD165 detector at the BL41-XU beamline of Spring-8 (Harima, Japan). Crystals were frozen either in liquid nitrogen directly or by using paratone as a cryoprotectant. Diffraction data were processed and integrated using HKL2000 (23). The unit cell parameters, space group and other data collection statistics are summarized in Supplementary Table 1. The crystal structure of hZxADAR1/d(TCGGCCG)2 (PDB ID 1QBJ) was used for the initial model of the other complex structures. Refinement and model building were performed by CNS (24) and O (25), respectively. The refinement statistics are summarized in Supplementary Table 1. The crystal structure of hZxADAR1/d(TCGCCCG):d(TCGGGCG) was not refined because of ambiguity in base assignment. All figures were drawn using Molscript, Raster3D and Pymol (26,27, http://www.pymol.org). The structural superposition was performed using the LSQKAB CCP4 program (28).

**RESULTS AND DISCUSSION**

**Structure determination**

The crystal structures of hZxADAR1 complexes with three non-CG-repeat dsDNAs, (dT(CACGTG))2, (dTCGTACG)2 and (dT(GCGCGG))2.
and d(TCGGCCG)₂ were determined at resolutions of 2.2, 2.5 and 2.7Å, respectively (Supplementary Table 1). The simulated annealed omit maps contoured at 3σ near the altered sequences confirmed that current structural information represents each non-CG-repeat DNA (Supplementary Figure 1). In all three structures, the deoxythymidine overhangs at the 5’ end of the oligonucleotides were not modeled due to their weak electron densities, and therefore, are not mentioned in current study. There are three hZZADAR1 domains, assigned as chains A, B and C, and three DNA strands, chains D, E and F, in one asymmetric unit. Chains D and E form the Z-DNA duplex, and chain F forms a duplex with chain F in another asymmetric unit that is related by crystallographic 2-fold symmetry. Each DNA strand is bound to one hZZADAR1 domain. The protein/DNA complex made up of the chain C and chain F pair was used for structural analyses unless specified otherwise because its average temperature factor is the lowest among the three complexes in the same asymmetric unit.

**Overall structures**

Overall, the hZZADAR1 domains bound to the three different Z-DNAs used in this study have almost identical structures to that of hZZADAR1 bound to d(CGCGCGC)₂ (14). hZZADAR1 has an αβ topology containing a three-helix bundle flanked on one side by a twisted antiparallel β sheet. The root mean square deviations (RMSDs) between hZZADAR1 bound to d(CGCGCG)₂ and hZZADAR1 bound to d(CACGTG)₂, d(CGTACG)₂ or d(CGGCCG)₂ are 0.52, 0.19 and 0.19Å, respectively, when calculated using the 64 Cα atoms of hZZADAR1 (Figure 1A). The three Z-DNAs bound to hZZADAR1 are all in the Z-conformation with alternating anti- and syn-glycosidic bonds regardless of their sequence (Figure 1B). With the exception of purines G3 and pyrimidines C4 of d(CGCCCG)₂ that adopt the anti and syn conformations, respectively, all other purines and pyrimidines are in the syn and anti conformations, respectively (Figure 1B). The calculated double-stranded DNA parameters are within the range of a Z conformation of DNA (Supplementary Table 2). For Z-DNAs complexed with hZZADAR1, the RMSD values between d(CGCGCGC)₂ and other DNAs, d(CACGTG)₂, d(CGTACG)₂ and d(CGGCCG)₂ were 0.28, 0.33 and 0.56Å, respectively, when calculated with 63 DNA backbone atoms of chain F for each DNA structure (Figure 1B and Supplementary Table 3).

**Interactions between hZZADAR1 and non-CG-repeat Z-DNAs**

The structural analyses of hZZADAR1, mZZADAR1 and yabZZADAR1 bound to the Z conformation of d(CGCGCGC)₂ revealed well-conserved interactions with the Zs domains (10,11,14). Zs domains with a winged helix–turn–helix motif bind to Z-DNA in a conformation-specific manner. The residues in the recognition helix (z3) and in the wing play critical roles in binding Z-DNA through direct or water-mediated hydrogen bonds and van der Waals interactions (Figure 2). On Z-DNA, one continuous surface composed of the sugar-phosphate backbone of Z-DNA is mostly involved in protein contact.

Similarly, in the crystal structures of hZZADAR1 bound to the three non-CG-repeat dsDNAs the residues located in z3 and the wing mostly make contact with the Z-DNA backbones either directly or through water-mediated interactions (Figure 2). When the DNA-binding surfaces of the hZZADAR1 domains bound to the four Z-DNAs are compared, their curvatures and surface charge distributions are nearly identical (Figure 3). These results, together with the limited structural alterations found in hZZADAR1 domains, strongly demonstrate that the overall binding mode of hZZADAR1 to Z-DNA are well conserved regardless of the sequence of the bound Z-DNA.

The number and type of hZZADAR1 residues contacting bases on each DNA vary, mainly because some of the important residues that were identified as the DNA-contacting residues in hZZADAR1/d(CGCGCGC)₂ (14) are disordered (Figure 2). However, it does not appear that variations in protein/DNA contacts are related to the sequence of the Z-DNA, since the number of contacting residues is not the same, even among the three hZZADAR1 domains in one asymmetric unit of each ZZZADAR1/DNA crystal. For example, in most structures, the roles of Arg174 and Thr191 are neither well defined nor involved in DNA contact. In hZZADAR1/d(CGCGCGC)₂, Arg174 was shown to form a direct hydrogen bond to a phosphate atom and a water-mediated hydrogen bond to the ribose ring (14). However, in seven of the nine hZZADAR1 domains used in this study, the amino groups of Arg174 are not modeled due to their weak electron densities (Figure 2). In the case of Thr191, no DNA interaction has been found. Similarly, Lys169 and Lys170 are only in contact with DNA in some structures (Figure 2). In contrast, Asn173, Tyr177, Pro192, Pro193 and Trp195 of hZZADAR1 contribute to the recognition of Z-DNA in all cases. Asn173 and Tyr177, located in the recognition helix (z3), recognize the phosphate backbone of Z-DNA through direct or water-mediated hydrogen bonds as observed in hZZADAR1/d(CGCGCGC)₂ (14). Likewise, Pro192 and Pro193 in the wing interact with Z-DNA through van der Waals interactions. In some cases, Trp195 makes a water-mediated hydrogen bond to a phosphate, but its major role seems to be supporting Tyr177 via the hydrophobic edge-to-face interaction which stabilizes the interactions between Tyr177 and the bases in the N4 position (Figure 2). Moreover, some coordinated waters that are well defined and mediate hydrogen bonds between protein and DNA in the crystal structure of hZZADAR1/d(CGCGCGC)₂ are not found in some structures.

The decrease in protein/DNA interactions in the three complex structures compared to hZZADAR1/d(CGCGCGC)₂ can be explained in part by the decrease in diffraction resolution. However, it seems that protein–DNA interactions are not all affected by diffraction resolution since well-defined interactions are consistently observed in all three structures despite their resolution differences. For example, the electron density of the well-defined hydrogen bonds between Tyr177 and the phosphate group of G3 are very clear even in the case of hZZADAR1/d(CGCGCGC)₂.
whose structure was determined at 2.7 Å resolution (Supplementary Figure 2). Conversely, it is suspected that some of the unseen protein/DNA interactions from these three Z-DNA structures are neither strong nor essential for Z-DNA recognition. From these results, it can be hypothesized that some of the residues previously identified as Z-DNA binders are not indispensable for Z-DNA binding but that they have auxiliary roles in DNA binding and probably produce tighter binding. However, we cannot rule out the possibility that crystal packing forces may have affected or destabilized some of the interactions between hZzADAR1 and Z-DNA.

In the Zz/d(CGCGCG) structure as well as in the current crystal structure of hZzADAR1/d(CATGCG)2, the tyrosine residue of the recognition helix (α3) has a unique role in recognizing the C-8 carbon of the syn deoxyguanosine at the forth position (G4) via a CH–π interaction (10,11,14; Figure 2A). In the case of Zz/d(CGTACG)2, the deoxyadenosine (A4) also adopts the syn conformation, which was very similar to that observed in previous studies (Figure 2B). More interesting is the observation that hZzADAR1 stabilizes the syn conformation of deoxycytidine at the forth position (C4) of d(CGGCCG)2 (Figure 2C). Generally, the syn conformation is not
Figure 2. The protein-DNA interactions in three different Zα-non-alternating CG ZDNA complexes. (A) hZαADAR1/d(CACGTG)2, (B) hZαADAR1/d(CGTACG)2 and (C) hZαADAR1/d(CGGCCG)2. One representative Zα/DNA complex (chains C and F) of the three complexes in one asymmetric unit is drawn as a tubular ribbon diagram (left). The three complexes in the asymmetric unit are then shown as schematic diagrams (left to right). These are chains A and D, chains B and E, and chains C and F, respectively. In the schematic diagrams, the amino acids identified as the DNA-contacting residues in the structure of the hZαADAR1/d(CGCCCG)2 complex are marked by boxes. Disordered residues are indicated by dotted boxes. Hydrogen bonds are represented by dotted lines and van der Waals contacts by open circles. The CH–π interaction between the conserved Tyr residue and syn deoxynucleoside is indicated by filled circles. Waters are shown as gray circles. In the tubular ribbon diagrams, the same residues used in the schematic diagrams are drawn as stick models and labeled. The DNA backbones and labeled bases are shown as red and gray stick models, respectively. Water molecules are shown as green spheres. Hydrogen bonds are drawn as dashed lines.
favored for pyrimidine nucleotides unless they are modified (3,4). However in the crystal structure of hZ\textsubscript{ADAR1}.d(CGGCCG)\textsubscript{2}, the \textit{syn} conformation of deoxyctydine is stabilized by the CH–p interaction between the C-5 and C-6 carbons of C4 and the \pi orbital of the tyrosine ring (Figure 2C). These data show that the binding mode of hZ\textsubscript{ADAR1} observed for d(CGCCCG)\textsubscript{2} is also a template for non-CG-repeat Z-DNAs containing non-APP repeat sequences or A-T base pairs. As with the earlier structure, there are no sequence-specific interactions between Z\textsubscript{a} and Z-DNA. These results reinforce the idea that the binding of Z\textsubscript{a} to Z-DNA is sequence-independent but conformation specific.

**Structural variations between free and hZ\textsubscript{ADAR1}-bound Z-DNAs**

The crystal structures of d(CGCCCG)\textsubscript{2}, d(CACGTG)\textsubscript{2}, d(m\textsubscript{5}CGTAm\textsubscript{5}CG)\textsubscript{2} and d(m\textsubscript{6}CGGC\textsubscript{m}CGC\textsubscript{m}CG)\textsubscript{2} were used to represent Z-DNAs free of protein binding (Supplementary Table 3; 2,8,29,30), and they have been compared with the hZ\textsubscript{ADAR1}-bound Z-DNA structure. In order to stabilize and crystallize Z-DNA with non-APP or A-T base pairs, base modification is used or it is necessary to add cations such as metal ions or polyamines. The 5 position of cytosine was methylated for the crystallization of Z-form d(CG TACG)\textsubscript{2} and d(CGGCCG)\textsubscript{2} (8,29), and spermine was added for the crystallization of Z-form d(CGCCCG)\textsubscript{2} and d(CACGTG)\textsubscript{2} (2,30). When the protein-free and hZ\textsubscript{ADAR1}-bound Z-DNAs were compared by superimposing each DNA pair using 63 DNA backbone atoms, RMSDs of d(CGCCCG)\textsubscript{2}, d(CACGTG)\textsubscript{2}, d(CGTACG)\textsubscript{2} and d(CGGCCG)\textsubscript{2} pairs were 0.89, 0.75, 0.57 and 0.61 Å, respectively (Figure 4 and Supplementary Table 3). Regardless of their sequence, the main structural differences between the free and bound Z-DNAs were found in the helical rise: when bound to hZ\textsubscript{ADAR1} it increased in the N3pN4 step, but decreased in the N2pN3 and N4pN5 steps (Supplementary Table 2). However, the distance changes in the N1pN2 and N5pN6 steps occurred regardless of Z\textsubscript{a} binding (Supplementary Table 2).

Structural variations between protein-free and hZ\textsubscript{ADAR1}-bound Z-DNAs were compared directly by plotting the distance between two corresponding atoms in Z-DNAs along the DNA backbone atoms (Figure 5A). In this manner, the structures of non-CG-repeat Z-DNAs and CG-repeat Z-DNA were also analyzed (Figure 5B–D). Structural deviations are plotted for all comparisons of protein-free and Z\textsubscript{a}-bound Z-DNA sequences. The largest structural variation between protein-free and hZ\textsubscript{ADAR1}-bound Z-DNAs was detected in the two phosphates at the N2pN3 and N4pN5 phosphodiester steps, respectively. These results were expected because the Z\textsubscript{a} conformation of the GpC
phosphodiester step is preferred when Z-DNA forms a complex with hZ\textsubscript{ADAR1} due to the specific interaction between the phosphate groups of DNA and the charged residues of Z\textsubscript{α}, whereas protein-free Z-DNA can have two alternative conformations, Z\textsubscript{I} and Z\textsubscript{II} (14,31). The difference between two corresponding atoms of the compared Z-DNAs is >2.5 Å when the protein-free Z-DNA is in the Z\textsubscript{II} conformation, and it is <0.5 Å when the protein-free Z-DNA is in the Z\textsubscript{I} conformation (Figure 5A). It is interesting that the current structures revealed that hZ\textsubscript{ADAR1}-bound Z-DNAs do not absolutely have the Z\textsubscript{I} conformation. Because ionic interactions between the phosphates at the N5 position and the charged residues, Lys170 and Arg174, are absent or weak in d(CGTACG)\textsubscript{2} and d(CG GCCG)\textsubscript{2}, the phosphodiester conformation of the N4pN5 step is not a typical Z\textsubscript{I} conformation (Figures 1B, 5C and D). As a result, in both Z-DNAs, the phosphates at the N5 position still show structural deviation from that of d(CG CGCG)\textsubscript{2} in hZ\textsubscript{ADAR1}-bound structures (sky blue lines in Figure 5C and D). Specifically, the N4pN5 phosphodiester step of d(CG GCCG)\textsubscript{2} adopts an intermediate conformation between Z\textsubscript{I} and Z\textsubscript{II} in the Z\textsubscript{α}/DNA complex.

It is well known that there is no significant sequence-dependent conformational alteration in the Z-DNA backbone structure, although base-packing parameters vary in a sequence-dependent manner (2,3,8,30–32). However, when corresponding atoms of each DNA pair are compared, differences near all of the phosphate groups are notable although the extent varies depending on their positions (Figure 5B–D). However, the differences near the phosphate groups of each DNA pair are reduced when the DNAs are bound to hZ\textsubscript{ADAR1} (Supplementary Table 3 and Figure 5) except in the case of N3pN4 in comparing d(CG GCCG)\textsubscript{2} and d(CG GCCG)\textsubscript{2}. The reduction in structural alteration of the phosphate groups of Z\textsubscript{α}-bound DNA is probably due to structural restraint enforced by Z\textsubscript{α} binding. These results strongly support the idea that the preformed binding pocket of Z\textsubscript{α} functions as a mold in recruiting various Z-DNAs, and it freezes the backbone conformations of Z-DNAs. As a result, structural variations of Z-DNAs that are caused mostly by conformational heterogeneity near the phosphate groups are reduced upon their binding into the Z\textsubscript{α} cleft.

![Figure 5. Structural deviation of each DNA backbone atom, comparing free Z-DNA and Z\textsubScript{ADAR1}-bound Z-DNA in both non-CG-repeat and CG-repeat Z-DNAs. The distances in angstrom between two backbone atoms in the same position of each paired DNA were plotted against the DNA backbone atoms. The structures were compared in the same way as shown in Figure 4. (A) The backbone atoms of Z-DNAs with CG GCCG, CAC GTG, CGTACG and CGGCCG sequences in free- and hZ\textsubScript{ADAR1}-bound forms were compared. (B) CAC GTG, (C) CGTACG and (D) CG GCCG DNAs in both free and hZ\textsubScript{ADAR1}-bound forms were compared with free- and hZ\textsubScript{ADAR1}-bound CG-repeat Z-DNA (CG GCCG).](https://academic.oup.com/nar/article-abstract/37/2/629/2410165)
Z-DNAs containing non-APP sequences or A-T base pair(s) have intrinsic structural instability and an increased solvent-exposed surface since base pairs are buckled out of the base-pair plane and protrude into the major groove (3,8,30,31). These structural features are still observed in hZADAR1-bound Z-DNAs (Supplementary Table 2). For example, d(CGTACG)2 and d(CGCGCG)2 reveal a huge buckle and decreased stacking interactions, whether bound to Zz or not (Figure 4 and Supplementary Table 2). Therefore, it is thought that the base packing pattern and structural instability of non-CG-repeat Z-DNAs are always maintained, whereas the phosphate backbone conformations are restrained by the Z-DNA-binding pocket of hZADAR1. Overall, additional structural instability of the Z conformation caused by non-CG-repeat sequences did not significantly affect the maintenance of the Z confirmation of DNAs in their complexes with Zz. These results suggest that more energy is gained upon Zz binding than is needed to overcome the structural instability of non-CG-repeat Z-DNAs.

CONCLUSIONS

Our structural data strongly support the idea that hZADAR1 recognizes Z-DNA in a conformation-specific manner, but not in a sequence-specific manner. The structures of hZADAR1 complexed with three different non-CG-repeat double-stranded Z-DNAs (with non-APP sequences or A-T base pairs) reveal that hZADAR1 binds and stabilizes the Z conformation of DNA via a similar binding mode to that of the Zz/d(CCGCGG)2 complex, regardless of sequence context. Most notably, the CH–π interaction was observed between Tyr177 and the cytosine in the syn conformation. While structures of phosphate backbones in free and hZADAR1-bound states display some variation near each phosphate group, hZADAR1 does not exert a profound effect on the Z-DNA base pair parameters induced by incorporation of non-APP and A-T base pairs. Irrespective of the heterogeneity in sequence and structure of non-CG-repeat Z-DNAs, Zz recognizes the DNA backbone atoms and imposes structural restraint through the core residues located on the DNA-binding surface. It has been suggested that different Z-DNA-binding proteins can bind to chromosomal or foreign DNAs and take part in essential biological processes. In this context, the Zz domain is expected to recognize and stabilize stretches of DNA in the Z-DNA conformation. Our results provide a molecular basis for understanding how the Zz proteins recognize and stabilize Z-DNAs in various sequence contexts.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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