Thermodynamic Model for B-Z Transition of DNA Induced by Z-DNA Binding Proteins

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Abstract: Z-DNA is stabilized by various Z-DNA binding proteins (ZBPs) that play important roles in RNA editing, innate immune response, and viral infection. In this review, the structural and dynamics of various ZBPs complexed with Z-DNA are summarized to better understand the mechanisms by which ZBPs selectively recognize (CG)-repeat DNA sequences in genomic DNA and efficiently convert them to left-handed Z-DNA to achieve their biological function. The intermolecular interaction of ZBPs with Z-DNA strands is mediated through a single continuous recognition surface which consists of an $\alpha_3$ helix and a $\beta$-hairpin. In the ZBP-Z-DNA complexes, three identical, conserved residues (N173, Y177, and W195 in the $Z\alpha$ domain of human ADAR1) play central roles in the interaction with Z-DNA. ZBPs convert a 6-base DNA pair to a Z-form helix via the B-Z transition mechanism in which the ZBP first binds to B-DNA and then shifts the equilibrium from B-DNA to Z-DNA, a conformation that is then selectively stabilized by the additional binding of a second ZBP molecule. During B-Z transition, ZBPs selectively recognize the alternating (CG)$_n$ sequence and convert it to a Z-form helix in long genomic DNA through multiple sequence discrimination steps. In addition, the intermediate complex formed by ZBPs and B-DNA, which is modulated by varying conditions, determines the degree of B-Z transition.

Keywords: Z-DNA; DNA-protein interaction; B-Z transition; Z-DNA binding protein

1. Introduction

Left-handed Z-DNA is a higher energy conformation than right-handed B-DNA. Z-DNA was first found in a polymer of alternating (CG)$_n$ DNA duplexes observed in high salt conditions [1]; its crystal structure was reported in 1979 [2]. The Z-DNA helix is built from (CG)-repeats, with the dC in the anti-conformation and the dG in the unusual syn-conformation, which causes the backbone to follow a zigzag path [3,4]. Z-DNA can also be stabilized by negative supercoiling generated behind a moving RNA polymerase during transcription [5].

A distinct biological function of Z-DNA is suggested by the discovery of various Z-DNA binding proteins (ZBPs). Double stranded (ds) RNA deaminase 1 (ADAR1) deaminates adenine in pre-mRNA to yield inosine, which codes as guanine [6–8]. ADAR1 has two left-handed Z-DNA binding domains (ZBDs), $Z\alpha$ and $Z\beta$, at its NH$_2$-terminus [7,9]. High binding affinity of this $Z\alpha$ domain to Z-DNA was shown by a band-shift assay and confirmed by CD and Raman spectroscopic measurement [10–12]. The DNA-dependent activator of IFN-regulatory factors (DAI; also known as ZBP1 or DLM-1) also contains two tandem ZBDs (Z$\alpha$ and Z$\beta$) at the NH$_2$-terminus, such as ADAR1 [13,14]. It has been shown that ZBDs regulate the localization of DAI and its association with stress granules [15,16]. All poxviruses have a gene called E3L that consists of two domains: An N-terminal ZBD and a C-terminal RNA binding domain [17,18]. This ZBD shows sequence homology to the Z$\alpha$ domains.
found in human ADAR1 and in the DAI of mammals (Figure 1a). The Z-DNA binding affinity of E3L protein is essential for pathogenesis in the poxviruses [17–19]. The RNA-dependent protein kinase (PKR) plays an important role in the innate immune response against viral infections by recognizing dsRNA in the cytosol [20–22]. In fish species, a functional analogue of PKR, PKZ contains two ZBDs instead of dsRNA binding domains [23–26]. Similar to PKR, the phosphorylation function of PKZ is activated by Z-DNA binding [24].

These biological data, along with the results of more recent structural studies of Z-DNA induced by various ZBPs, have provided insights into Z-DNA recognition and ZBP-induced B-Z transition carried out by the innate immune response, viral infection, and RNA editing that are influenced by the nature of the Z-DNA. Crystallographic and NMR studies have provided detailed three-dimensional (3D) structures and dynamic information of various ZBPs complexed with Z-DNA. The structural and dynamic data summarized in this review have yielded a rich understanding of the mechanisms by which ZBPs selectively recognize the (dCG)-repeat DNA sequences in genomic DNA and efficiently convert them to left handed Z-DNA to achieve their biological function.

Figure 1. (a) Multiple sequence alignment of ZBPs: hZ\(_{\text{ADAR1}}\), hZ\(_{\text{\beta ADAR1}}\), human ADAR1; mZ\(_{\text{ADAR1}}\), mZ\(_{\text{\beta ADAR1}}\), murine ADAR1; hZ\(_{\text{DAI}}\), hZ\(_{\text{\beta DAI}}\), human DAI; mZ\(_{\text{DAI}}\), mZ\(_{\text{\beta DAI}}\), murine DAI; yabZ\(_{\text{E3L}}\). Yaba-like disease virus E3L; vZ\(_{\text{E3L}}\), vaccinia virus E3L; orfZ\(_{\text{E3L}}\), orf virus E3L; lsZ\(_{\text{E3L}}\), lumpy skin disease virus E3L; spZ\(_{\text{E3L}}\), swinepox virus E3L; caZ\(_{\text{PKZ}}\), caZ\(_{\text{PKZ}}\); goldfish PKZ; drZ\(_{\text{PKZ}}\), drZ\(_{\text{PKZ}}\); zebrafish PKZ. Numbering and secondary structural elements for hZ\(_{\text{ADAR1}}\) and hZ\(_{\text{\beta ADAR1}}\) are shown above the sequence. Yellow and gray bars indicate residues important for Z-DNA recognition and protein folding, respectively. (b) Overview of the hZ\(_{\text{ADAR1}}\) domain bound to left-handed Z-DNA (PDB id: 1QBJ) [7]. (c) View of the DNA recognition surface of hZ\(_{\text{ADAR1}}\) (PDB id: 1QBJ) [7]. The green lines indicate the H-bonding interactions. In (b,c), the backbone structure of hZ\(_{\text{ADAR1}}\) domain and Z-DNA duplex, d(TCCGCGCG)_2, are represented by the green ribbon and element-based stick presentation, respectively.
2. Crystal Structures of ZBPs Complexed with DNA Duplexes

2.1. hZ\textsubscript{α}ADAR1-Z-DNA Complex

In 1999, Alexander Rich and his colleagues first reported the crystal structure of the Z\textsubscript{α} domain of human ADAR1 (hZ\textsubscript{α}ADAR1) complexed with a six-base-pair (bp), double-stranded (ds) DNA fragment d(TCGCGCG)\textsubscript{2} [7]. The monomeric hZ\textsubscript{α}ADAR1 domain binds to one strand of the palindromic dsDNA, in which the conformation of the DNA substrate is very similar to the canonical Z-DNA structure. (Figure 1b) [7]. A second monomer binds to the opposite strand of DNA yielding two-fold symmetry with respect to the DNA helical axis (Figure 1b) [7]. The hZ\textsubscript{α}ADAR1 domain has a compact \(\alpha/\beta\) architecture containing a three-helix bundle (\(\alpha_1\) to \(\alpha_3\)) and twisted antiparallel \(\beta\) sheets (\(\beta_1\) to \(\beta_3\)) (Figure 1b). The arrangement of a \(\beta\)-hairpin with hydrogen-bonds (H-bonds) between the \(\beta_2\) (L185–A188) and \(\beta_3\) strands (P193–I197) is a common feature of helix-turn-helix (HTH) proteins with \(\alpha/\beta\) topology [7]. Aliphatic residues from the three helices, together with the W195 in strand \(\beta_3\), form a hydrophobic core [7]. The NMR studies reported that the free hZ\textsubscript{α}ADAR1 protein in the solution adopts a similar fold in its complex structure [9,27].

The contact of hZ\textsubscript{α}ADAR1 with the Z-DNA strand is mediated through a single continuous recognition surface, which consists of residues from an \(\alpha_3\) helix and a \(\beta\)-hairpin (\(\beta_2\)-loop-\(\beta_3\), also called a \(\beta\)-wing) (Figure 1c) [7]. The electrostatic interactions in the complex are made between K169, K170, N173, R174, and Y177 in an \(\alpha_3\) helix as well as between T191 and W195 in a \(\beta\)-hairpin and five consecutive phosphate-backbones of Z-DNA (Figure 1c) [7]. K169 and N173 form the direct and water-mediated H-bonds to the dC3pdG4 and dG2pdC3 phosphates, respectively (Figure 2a) [7]. The N173A mutant displays the most dramatic decrease in Z-DNA binding affinity, suggesting that it plays an important role in the Z\textsubscript{α} function [27,28]. Similarly, the K169A mutant also has a significantly lower Z-DNA binding affinity than a wild-type protein [27,28]. K170 forms direct H-bonds to the dG4pdC5 and dC5pdG6 phosphates (Figure 2a) [7]. The K170A mutant binds to Z-DNA with a lower affinity than wild-type protein, but better than the K169A and N173A mutants [27,28]. Interestingly, R174 and T191 bind to the furanose oxygens of dG6 and dG2, respectively (Figure 2a) [7]. However, the R174A and T191A mutations have little effect on the B-Z transition activities of hZ\textsubscript{α}ADAR1 [28].

In addition to polar interactions, the aromatic ring of Y177 and the side-chains of P192 and P193 make the important van der Waals interactions with Z-DNA (Figure 2a) [7]. Interestingly, Y177 displays CH–π interaction with the C6 position of dG4 (Figure 2a) [7]. The Y177A mutant, which is unable to form both H-bonding and hydrophobic interactions, exhibits a significantly low Z-DNA binding affinity [27]. The Y177I, and Y177F mutants, which are capable only of hydrophobic interactions, could bind better to Z-DNA than Y177A, but still worse than wild-type protein [17,27,28]. Furthermore, the aromatic ring of W195, which forms a water-mediated H-bond to the dG2pdC3 phosphate, is almost perpendicular to Y177 and positioned in the center of the hydrophobic core (Figure 1c) [7]. The W195F mutant has 2-fold lower B-Z transition activity than wild type protein [28].
Non-CG-repeat sequences, such as d(CACGTG) (Figure 2a,b) [7,29]. K43 and Q47 form direct or water-mediated H-bonds to three phosphates of the backbone of Z-DNA. Mutagenesis studies of these residues have shown that all three residues are important for Z-DNA-binding by ZαADAR1, with the addition of a C-terminal α4 helix (see sequence in Figure 1a) [35]. Superposition of the hZβADAR1 with the hZαADAR1 structure reveals that A327, A332, and I335 (corresponding to K169, R174, and Y177 in hZαADAR1, respectively) did not perform H-bonding to the backbone of Z-DNA. Mutagenesis studies of these residues have shown that all three residues are important for Z-DNA-binding by Zα proteins [17,27,28]. This study suggested that hZβADAR1 is unable to interact with nucleic acids in a manner similar to that seen in the hZαADAR1-Z-DNA complex [35]. Instead, this region participates in self-association protein–protein interactions [35].

2.2. mZαDLM1-Z-DNA Complex

The crystal structural study reported that hZαADAR1 is also able to bind to 6-bp Z-DNA duplexes with non-CG-repeat sequences, such as d(CACGTG)12, d(GTACGG)12, and d(CCAGCG)12 [34]. In these structures, N173, Y177, P192, P193, and W195 contribute to the recognition of Z-DNA-like CG-repeat DNA [34]. However, R174 and T191 did not show intermolecular interaction with Z-DNA in most structures [34]. Similarly, K169 and K170 are only in contact with Z-DNA within some structures of these complexes [34]. Thus, these four residues might play an important role in the sequence discrimination step for the B-Z transition of DNA.

The second Z-DNA binding domain of human ADAR1 (hZβADAR1) adopts a winged-HTH fold like hZαADAR1, with the addition of a C-terminal α4 helix (see sequence in Figure 1a) [35]. Superposition of the hZβADAR1 with the hZαADAR1 structure reveals that A327, A332, and I335 (corresponding to K169, R174, and Y177 in hZαADAR1, respectively) did not perform H-bonding to the backbone of Z-DNA. Mutagenesis studies of these residues have shown that all three residues are important for Z-DNA-binding by Zα proteins [17,27,28]. This study suggested that hZβADAR1 is unable to interact with nucleic acids in a manner similar to that seen in the hZαADAR1-Z-DNA complex [35]. Instead, this region participates in self-association protein–protein interactions [35].

Figure 2. Protein residues involved Z-DNA/Z-RNA interactions in (a) hZαADAR1–dT(CG)3 [7], (b) mZαDLM1–dT(CG)3 [29], (c) yabZαE3L–dT(CG)3 [30], (d) caZαPKZ–dT(CG)3 [31], (e) hZβADAR1–dT(CG)3 [32], and (f) hZαADAR1–rU(CG)3 complexes [33]. Intermolecular H-bonds and van der Waals contacts are indicated by solid lines and open circles, respectively. The water molecules in key positions within the protein–DNA interface are indicated by ovals.
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The structural differences between the two domains are found in the α1-β1 loop and the β-hairpin [29]. The β-hairpin of mZαDLM1 is two residues shorter than that of hZαADAR1 (Figure 1a), indicating that the β-hairpin is apparently tolerant of greater sequence variability than the α3 helix without loss of function [29].

2.3. yabZαE3L-Z-DNA Complex

The Zα domain of the Yaba-like disease virus E3L (yabZαE3L) stabilizes the Z-DNA conformation in a manner similar to that of hZαADAR1 and mZαDLM1 [30], although it shares only 26% sequence identity with hZαADAR1 (Figure 1a). The crystal structural study revealed that two yabZαE3L domains are found in the asymmetric unit, each bound to one strand of double-stranded DNA in the Z-conformation [30]. The intermolecular interaction of one asymmetric unit with Z-DNA is summarized in Figure 2c [30]. Three residues, N47, Y51, and W69 (corresponding to N173, Y177, and W195 in hZαADAR1), play central roles in the interaction with Z-DNA, as with other members of the Zα family (Figure 2c). K43, K44, and Q48 also participate in DNA recognition via direct or water-mediated H-bonds to the phosphate backbone of Z-DNA (Figure 2c).

2.4. caZαPKZ-Z-DNA Complex

The Zα domain of PKZ from Carassius auratus (caZαPKZ), which shows limited identity with other ZBPs (28% for hZαADAR1, 20% for hZαDAI, and 22% for yabZαE3L, respectively), is able to convert d(CG)-repeat DNA from B-DNA to Z-DNA [36,37]. The interaction between caZαPKZ and Z-DNA is mediated by five residues in the α3 helix and four residues in the β-hairpin, similar to other Zα proteins (Figure 2d) [31]. Unlike the positively charged Lys or Arg in other ZBPs, the S35 in the α3 helix forms electrostatic interaction with the dC3pdG4 phosphate (Figure 2d) [31]. Interestingly, K56 of caZαPKZ interacts not only with dC1pdG2 but also with dT0pdC1 (Figure 2d) [31], whereas a polar residue, like Ser or Thr at the corresponding position in other mammalian ZBPs, could not form these interactions (Figure 2). Generally, the B-Z transition activity by ZBPs was decreased when the ionic strength was increased. Surprisingly, the reduction of the B-Z transition rate is more severe in caZαPKZ than in hZαADAR1, suggesting that the effect of charge-charge interactions on B-to-Z transition activity plays a more critical role in the case of caZαPKZ [31].

2.5. hZβDAI-Z-DNA Complex

The second ZBD of human DAI (hZβDAI) was also shown to bind Z-DNA based on its binding specificity for Z-DNA and its ability to convert B-DNA to Z-DNA [16]. Although hZβDAI also has α/β topology with three helices packed against three β-stands, like other ZBPs, hZβDAI has a 310 helix at the N terminus of α3, instead of the long continuous α3 helix [32]. In the hZβDAI-Z-DNA complex, protein-DNA interactions are mediated by the most conserved core residues, N141, Y145, and W162 (corresponding to N173, Y177, and W195) [32]. However, except for those core residues, other interactions with Z-DNA seem to be different for hZβDAI (Figure 2) [32]. For example, K138, located in the region between K169 and K170 of hZαADAR1, forms an H-bond to the dC3pdG4 phosphate of one Z-DNA strand, whereas the two Lys residues of hZαADAR1 contact the 4 phosphate groups of Z-DNA (Figure 2) [32]. Interestingly, K138 spans the length of the Z-DNA molecule and interacts with the dC5pdG6 phosphate on the opposite DNA strand (Figure 2e) [32]. An NMR study found that free hZβDAI has notable alterations in the α3 helix, the β-hairpin, and Y145 which are critical in Z-DNA recognition [38]. These results indicate that, unlike some other Zα domains, structural flexibility of hZβDAI is required for Z-DNA binding [38].

2.6. hZαADAR1-Z-RNA Complex

ADAR1 edits dsRNA in vitro at significantly higher levels when dsRNA contains the purine-pyrimidine repeat sequence in dsRNA [39]. The hZαADAR1 protein can bind to Z-RNA like
Z-DNA [40]. It was first reported that the crystal structure of hZαADAR1 complexed with 6-bp dsRNA, r(UCGCCG)₂ [33]. Interestingly, hZαADAR1 exhibited significantly different binding modes when bound to Z-RNA versus Z-DNA (Figure 2). First, in the Z-RNA binding conformation, Y177 showed H-bonding interaction with the rG2pC3 phosphate and the O2’ of rG2, whereas it H-bonded with only the dG2pdC3 phosphate in the Z-DNA binding structure (Figure 2) [7,33]. Second, in the Z-DNA binding structure, R174 showed a direct, water-mediated H-bonding interactions with the dC5pdG6 phosphate and the O4’ of dG6, respectively (Figure 2a) [7]. However, when binding to Z-RNA, a water-mediated H-bond with the rC5prG6 phosphate as well as an H-bond with the E171 side-chain were formed (Figure 2f) [33]. Third, in the Z-RNA binding conformation, H159 exhibited a distinct orientation due to a water-mediated H-bonding interaction with the K169 side-chain compared to the Z-DNA binding conformation [33]. Fourth, in the Z-RNA binding conformation, T191 showed H-bonding interaction with only the rC3prG4 phosphate, whereas it formed H-bonds with both the phosphate and the O4’ of dC3 in the Z-DNA binding structure (Figure 2) [7,33].

3. Molecular Mechanism of B-Z Transition of 6-bp DNA Induced by ZBPs

3.1. B-Z Transition of a 6-bp CG-Repeat DNA by hZαADAR1

NMR studies on the hZαADAR1-Z-DNA interaction first proposed the B-Z transition mechanism of a 6-bp DNA, d(CGCGCG)₂, by hZαADAR1, in which the hZαADAR1 plays two independent roles: (i) one molecule first binds to B-DNA and shifts the equilibrium from B-DNA to Z-DNA (BP to ZP, where B, Z, and P indicate B-DNA, Z-DNA, and protein); and (ii) the second molecule selectively binds to and stabilizes the Z-DNA conformation (Figure 3a) [41]. This study confirmed the existence of a one-to-one complex of Z-DNA and hZαADAR1 (ZP) by gel filtration chromatography, NMR dynamics data, and diffusion coefficient values as functions of the [P]₀/[N]₀ molar ratio, where [P]₀ and [N]₀ are the total concentrations of the hZαADAR1 and DNA, respectively [41]. It was found that the Z-DNA produced was half the total amount of the added hZαADAR1 when the [P]₀/[N]₀ ratio was ≤2 (that is Z t = 1/2[P]₀), where Z t is the total amount of the Z-DNA conformation (Figure 3b) [41]. To satisfy this relation, the BP and ZP complexes must exist as intermediate states with a correlation of [ZP] = [BP] (that is K_BZ,1 = [ZP]/[BP] ≈1). Based on this correlation, the observed exchange rate constant (k_ex) for the imino proton in the Z-DNA conformation could be expressed as a function of the [P]₀/[N]₀ ratio by the following Equation:

$$k_{ex} = k_{ex,ZP} + k_{ex,ZP_2} \frac{k_{ex,ZP} - k_{ex,ZP_2}}{(1 - \alpha)\chi} \left\{ 1 - \frac{1}{4(1 - \alpha)} \left( \frac{\chi}{2} - \frac{\chi^2}{4} \right) \right\}$$

(1)

where $k_{ex,ZP}$ and $k_{ex,ZP_2}$ are the exchange rate constants of the imino protons for the ZP and ZP₂ complexes, respectively, $\chi$ is the [P]₀/[N]₀ ratio, and $\alpha (= K_d,ZP_2/K_d,BP)$ is the ratio of the dissociation constants of BP and ZP₂ complexes [41]. The $k_{ex}$ dataset was fitted using Equation (1) to obtain the $\alpha$ value of $1.15 \times 10^{-2}$ (Table 1) [41].
Equation (1) could not be used to analyze the hydrogen exchange data of these DNA because the non-CG-repeat DNA did not satisfy the relation, $Z_t = 1/2[P]_t$. Instead, the observed $k_{ex}$ value for the imino proton in the B-DNA (not Z-DNA) conformation could be expressed as a function of the relative Z-DNA population $(f_Z = Z_t/[N]_t)$ by the following equation:

$$k_{ex} = k_{ex,B} + \frac{k_{ex,B} - k_{ex,Z}}{Z_t - 1} \left(1 + (K_{Z,B,1} - 1)f_Z - \sqrt{(1 + (K_{Z,B,1} - 1)f_Z)^2 - 4K_{Z,B,1}(1 - \alpha)f_Z(1 - f_Z)}\right)$$  \hspace{1cm} (2)

Table 1. Equilibrium constants for the ZBP-induced B-Z transition.

| ZBP       | DNA    | $\alpha$ | $K_{d,BP}$ (\mu M) | $K_{d,ZP2}$ (\mu M) | References |
|-----------|--------|----------|---------------------|----------------------|------------|
| hZ\alpha_{ADAR1} | d(CGCGCG) 2 | 1.15 × 10^{-2} | 0.1 | 144 | 29 ± 11 | [42] |
| hZ\alpha_{ADAR1} | d(CACGTG) 2 | 1.42 | 0.4 | 260 ± 87 | 180 ± 62 | [42] |
| hZ\alpha_{ADAR1} | d(CGCGCG) 2 | 13.9 | 6.3 | 400 ± 144 | 29 ± 11 | [42] |
| yabZ\alpha_{E3L} | d(CGCGCG) 2 | 0.154 | 1.02 | n.d. \textsuperscript{2} | n.d. \textsuperscript{2} | [43] |

\textsuperscript{1} $\alpha = K_{d,ZP2}/K_{d,BP}$; \textsuperscript{2} n.d.: not determined.

3.2. B-Z Transition of a 6-bp Non-CG-Repeat DNA by hZ\alpha_{ADAR1}

The hZ\alpha_{ADAR1} protein can also convert the B-form of non-CG-repeat DNA, d(CACGTG) 2 and d(CGCGCG) 2, to Z-form with lower activities compared to CG-repeat DNA, d(CGCGCG) 2 (Figure 3b) \cite{41,42}. Equation (1) could not be used to analyze the hydrogen exchange data of these DNA complexed with hZ\alpha_{ADAR1} because the non-CG-repeat DNA did not satisfy the relation, $Z_t = 1/2[P]_t$. Instead, the observed $k_{ex}$ value for the imino proton in the B-DNA (not Z-DNA) conformation could be expressed as a function of the relative Z-DNA population $(f_Z = Z_t/[N]_t)$ by the following equation:

$$k_{ex} = k_{ex,B} + \frac{k_{ex,B} - k_{ex,Z}}{Z_t - 1} \left(1 + (K_{Z,B,1} - 1)f_Z - \sqrt{(1 + (K_{Z,B,1} - 1)f_Z)^2 - 4K_{Z,B,1}(1 - \alpha)f_Z(1 - f_Z)}\right)$$  \hspace{1cm} (2)
where $k_{\alpha,B}$ and $k_{ex,BP}$ are the exchange rate constants of the imino protons for free B-DNA and the BP complex, respectively [42]. The NMR dynamics studies found that hZ$_{\alpha,ADAR1}$ binds to non-CG-repeat DNA with weak binding affinity through the $\alpha_3$ helix as well as through the loop-$\beta_1$-loop (151–158) and the $\alpha_3$-loop-$\beta_2$ regions (178–191) [46]. Then, the B-form helix of non-CG-repeat DNA duplexes can be converted to a Z-conformation via these multiple intermolecular interactions with hZ$_{\alpha,ADAR1}$ proteins [46]. These studies explained how hZ$_{\alpha,ADAR1}$ exhibited the sequence preference of d(CGCGCG)$_2$ > d(CACGTG)$_2$ > d(CGTACG)$_2$ during the B-Z transition [42,46]. First, the P binds to the B, with a sequence preference of d(CGCGCG)$_2$ > d(CACGTG)$_2$ > d(CGTACG)$_2$ [42], even though the structural features of these three DNA duplexes complexed with hZ$_{\alpha,ADAR1}$ are very similar to each other [34]. Second, the BP of d(CGCGCG)$_2$ and d(CACGTG)$_2$ convert to ZP. In d(CGTCAG)$_2$, however, this process is less efficient as a way of discriminating d(TA)-containing DNA sequences from alternating pyrimidine-purine sequences [42]. Third, the ZP of d(CGCGCG)$_2$ and d(CACGTG)$_2$ binds to the P and forms the stable ZP complex with a sequence preference of d(CGCGCG)$_2$ > d(CACGTG)$_2$, which acts as the third sequence discrimination step [42]. Taken together, it was suggested that hZ$_{\alpha,ADAR1}$ selectively recognizes the alternating d(CG)$_n$ sequence and then converts it to a Z-form helix in long genomic DNA through its multiple sequence discrimination steps [42,46].

3.3. B-Z Transition of a 6-bp DNA by yabZ$_{\alpha,E3L}$

The yabZ$_{\alpha,E3L}$ could efficiently change the B-form helix of the d(CGCGCG)$_2$ to left-handed Z-DNA like hZ$_{\alpha,ADAR1}$ (Figure 3c) [43]. In this study, because the B-Z transition activity of yabZ$_{\alpha,E3L}$ did not satisfy the relation, $Z_1 = 1/2[P]_n$, the observed $k_{ex}$ value for the imino proton in the Z-DNA conformation could be expressed by the following equation instead of Equation (1):

$$k_{ex} = k_{ex,ZP} + \frac{k_{ex,ZP} - k_{ex,ZP}}{K_{BZ,1}(1-\alpha)^2} \left[1 + \left(K_{BZ,1} - 1\right)f_z - \sqrt{(1 + (K_{BZ,1} - 1)f_z)^2 - 4K_{BZ,1}(1-\alpha)f_z(1-f_z)}\right]$$

(3)

The $k_{ex}$ dataset was fitted using Equation (3) to obtain the $\alpha$ value of 0.154 and the $K_{BZ,1}$ value of 1.02 (Table 1) [43]. This $K_{BZ,1}$ value means that yabZ$_{\alpha,E3L}$ and hZ$_{\alpha,ADAR1}$ have the same B-Z transition efficiency [43], which is consistent with their structural similarity in complexes with Z-DNA [7,30].

3.4. B-Z Transition of a 6-bp DNA by hZ$_{\beta,DAI}$

NMR studies have revealed that hZ$_{\beta,DAI}$ had significantly lower B-Z transition activity than hZ$_{\alpha,ADAR1}$ and yabZ$_{\alpha,E3L}$ (Figure 3c) [44]. In addition, the imino proton and $^{31}$P-NMR spectra of d(CGCGCG)$_2$ complexed with hZ$_{\beta,DAI}$ are completely different from those of the d(CGCGCG)$_2$-hZ$_{\alpha,ADAR1}$ complex [44]. These indicate that the base pair geometry and backbone conformation of the hZ$_{\beta,DAI}$-induced Z-DNA helix are significantly different from those of the Z-DNA-hZ$_{\alpha,ADAR1}$ complex, similar to their crystal structures [7,32]. The hydrogen exchange study of the d(CGCGCG)$_2$-hZ$_{\beta,DAI}$ complex found that the exchange rates of imino protons in B-DNA as well as Z-DNA conformations are not affected by complex formation [44]. In addition, diffusion optimized spectroscopy experiments confirmed that the Z-form of d(CGCGCG)$_2$ complexed with hZ$_{\beta,DAI}$ exhibited one major complex state (perhaps ZP$_2$), even at various $[P]_n/[N]_n$ [44]. Based on these results, they proposed the distinct B-Z transition mechanism where two molecules of hZ$_{\beta,DAI}$ initially bind directly to the B-form DNA and form the BP$_2$ complex; subsequently, there is a conformational change from BP$_2$ to ZP$_2$. (Figure 3a) [44].

3.5. B-Z Transition of a 6-bp DNA by caZ$_{\alpha,PKZ}$

The caZ$_{\alpha,PKZ}$ domain can convert the dsDNA, d(CGCGCG)$_2$ to Z-DNA with lower activity rates than hZ$_{\alpha,ADAR1}$ and yabZ$_{\alpha,E3L}$ (Figure 3d) [45]. Instead, caZ$_{\alpha,PKZ}$ exhibits full B-Z transition activity when binding to d(TCGCGCG)$_2$ (Figure 3d) [45]. This indicates that the H-bonding interaction of K56 with the dT0pdC1 phosphate plays an important role in the B-Z transition of DNA by caZ$_{\alpha,PKZ}$ [45]. In this study, instead of the $k_{ex}$ value, the $^1$H and $^{15}$N chemical shift changes ($\Delta^\delta_{obs}$) of amide protons
of caZαPKZ and relative Z-DNA population (fZ) were determined as the functions of [N]t and [P]t expressed by the following functions, respectively:

$$\Delta \delta_{\text{obs}} = \frac{[\text{BP}]}{[\text{P}]_t} \Delta \delta_{\text{B}} + \frac{[\text{ZP}]}{[\text{P}]_t} \Delta \delta_{\text{Z}}$$

(4)

$$f_Z = \frac{[\text{ZP}]+[\text{ZP}_2]}{[\text{P}]_t}$$

(5)

where $\Delta \delta_{\text{B}}$ and $\Delta \delta_{\text{Z}}$ are the $^1$H and $^{15}$N chemical shift differences of the B-DNA- and Z-DNA-bound forms relative to free form, respectively, [BP], [ZP], and [ZP2] are the concentration of the BP, ZP, and ZP2 complex states, respectively, which are described as:

$$[\text{BP}] = [\text{N}]_t \frac{K_{d,\text{BP}} [\text{P}]}{K_{d,\text{BP}} [\text{P}]+(1+K_{BZ,1})K_{d,\text{ZP}2} [\text{P}]+K_{BZ,1} [\text{P}]^2}$$

(6)

$$[\text{ZP}] = [\text{N}]_t \frac{K_{BZ,1} K_{d,\text{ZP}2} [\text{P}]}{K_{d,\text{BP}} K_{d,\text{ZP}2} + (1+K_{BZ,1})K_{d,\text{ZP}2} [\text{P}]+K_{BZ,1} [\text{P}]^2}$$

(7)

$$[\text{ZP}_2] = [\text{N}]_t \frac{K_{BZ,1} [\text{P}]^2}{K_{d,\text{BP}} K_{d,\text{ZP}2} + (1+K_{BZ,1})K_{d,\text{ZP}2} [\text{P}]+K_{BZ,1} [\text{P}]^2}$$

(8)

and [P] is the concentration of free caZαPKZ, solvable via the following cubic equation [45]:

$$[\text{P}]^3 + a[\text{P}]^2 + b[\text{P}] + c = 0$$

(9)

$$a = 2[\text{N}]_t - [\text{P}]_t + \left(1+\frac{1}{K_{BZ,1}}\right)K_{d,\text{ZP}2}$$

(10)

$$b = \left(1+\frac{1}{K_{BZ,1}}\right)K_{d,\text{ZP}2}([\text{N}]_t - [\text{P}]_t) + \frac{K_{d,\text{BP}} K_{d,\text{ZP}2}}{K_{BZ,1}}$$

(11)

$$c = -\frac{K_{d,\text{BP}} K_{d,\text{ZP}2} [\text{P}]_t}{K_{BZ,1}}$$

(12)

The closed-form solution of Equation (9) has been expressed by [45]:

$$[\text{P}] = -\frac{a}{3} + \frac{2}{3} \sqrt{\frac{a^2 - 3b \cos \frac{\theta}{3}}{3}}$$

(13)

where

$$\theta = \arccos \left(\frac{-2a^3+9ab-27c}{2\sqrt{(a^2-3b)^3}}\right)$$

(14)

In order to obtain accurate $K_d$ values, all $^1$H and $^{15}$N titration curves and the $f_Z$ data were globally fitted with Equation (4) and Equation (5), respectively. This approach successfully provides two binding constants, $K_{d,\text{BP}}$ and $K_{d,\text{ZP}2}$, not the relative ratio ($\alpha$) of these two constants as in previous studies.

At 10 mM NaCl, the global fitting gave a $K_{d,\text{BP}}$ and a $K_{d,\text{ZP}2}$ of 28 and 350 mM, respectively, and a $K_{BZ,1}$ of 0.87 (Table 2) [45]. As [NaCl] was increased, the $K_{d,\text{BP}}$ and $K_{d,\text{ZP}2}$ values became increased, but the $K_{BZ,1}$ value became smaller (Table 2) [45,47]. The NMR dynamics studies found that increasing the ionic strength interferes more with the association of ZP with caZαPKZ via intermolecular electrostatic interactions rather than the dissociation of ZP2 [45]. In addition, the global fitting method using Equation (4) also provides the $^1$H and $^{15}$N chemical shift differences between the free and the bound forms for both B-DNA ($\Delta \delta_{B}$) and Z-DNA binding ($\Delta \delta_{Z}$). At higher concentrations of NaCl, the B-DNA-bound state exhibited completely different results than at 10 mM NaCl, whereas the Z-DNA binding conformation was not affected by the change of ionic strength [45,47]. These results
meant that the B-DNA binding state of caZαPKZ exhibited distinct structural features under high and low salt conditions which might be related to reduced B-Z transition activity at higher [NaCl]. Taken together, these studies suggest that the intermediate complex formed by caZαPKZ and B-DNA can be used as a molecular ruler to measure the degree to which DNA transitions to the Z isoform [45,47].

| ZBP          | DNA         | pH  | [NaCl] | \(K_{BZ,1}\) | \(K_{d,BP}\) (µM) | \(K_{d,ZP2}\) (µM) | References |
|--------------|-------------|-----|--------|-------------|----------------|------------------|------------|
| caZαPKZ      | d(TCGCGCG)2 | 6.0 | 10 mM  | 0.87 ± 0.03 | 0.028 ± 0.017   | 0.345 ± 0.079    | [45]       |
| caZαPKZ      | d(TCGCGCG)2 | 6.0 | 100 mM | 0.19 ± 0.02 | 16.4 ± 0.8      | 8.76 ± 0.67      | [45]       |
| caZαPKZ      | d(TCGCGCG)2 | 6.0 | 250 mM | -0.01       | 64.1 ± 8.3      | 9.57 ± 0.85      | [47]       |
| caZαPKZ      | d(TCGCGCG)2 | 8.0 | 10 mM  | 1.18 ± 0.03 | 0.157 ± 0.021   | 0.129 ± 0.074    | [45]       |
| caZαPKZ      | d(TCGCGCG)2 | 8.0 | 100 mM | 0.18 ± 0.02 | 5.41 ± 0.66     | 2.41 ± 0.37      | [45]       |
| caZαPKZ      | d(CGCGCG)2  | 8.0 | 10 mM  | 0.11 ± 0.05 | 5.18 ± 2.43     | 1.79 ± 0.95      | [45]       |

4. Conclusions

Z-DNA is induced by various Z-DNA binding proteins (ZBPs) that play important roles in RNA editing, innate immune response, and viral infection. We summarized the structural and dynamics data of various ZBPs complexed with Z-DNA to understand the mechanisms by which ZBPs selectively recognize d(CG)-repeat DNA sequences in genomic DNA and efficiently convert it to left handed Z-DNA to achieve their biological function. The contact of ZBPs with Z-DNA strands mediated through a single continuous recognition surface consists of an α3 helix and a β-hairpin. In the ZBP-Z-DNA complexes, three conserved identical residues of ZBPs (N173, Y177, and W195 in hZα\textsubscript{ADAR1}) play a central role in interactions with Z-DNA. ZBPs convert a 6-bp DNA to a Z-form helix via a B-Z transition mechanism in which the ZBP first binds to B-DNA and then shifts the equilibrium from B-DNA to Z-DNA, a conformation that is then selectively stabilized by the additional binding of a second ZBP molecule. During B-Z transition, ZBPs selectively recognize the alternating d(CG)n sequence and then convert it to a Z-form helix in long genomic DNA through its multiple sequence discrimination steps. In addition, the intermediate complex formed by ZBPs and B-DNA, which is modulated by varying conditions, determines the degree of B-Z transition.

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