# Protein-Induced Sequence Diversity of Z-DNA

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Right-handed B-DNA is a major form of double-stranded DNA (dsDNA) and used for the structural support of genetic information. It has been also known that DNA can form diverse shapes either *in vivo* or *in vitro*. Among them, left-handed Z-DNA is one of the most well known non-B-DNA structures. Although the biological roles of Z-DNA *in vivo* have been elusive despite of intense studies since its discovery decades ago, recent finding of Z-DNA binding motif, Z$\alpha$, provides a promising outlook to elucidate the relevance of Z-DNA in biological systems. The Z$\alpha$ was first identified from an RNA editing enzyme; human dsRNA adenosine deaminase 1 (hADAR1) that has two Z$\alpha$-motif-containing-domains, $Z\alpha_{ADAR1}$ and $Z\beta_{ADAR1}$, at the N-terminal region.[1] Soon findings of several other proteins containing one or two Z$\alpha$ motifs have been followed.[1-3] The $Z\alpha_{ADAR1}$, the first N-terminal Z$\alpha$ domain from human ADAR1, has been used as a tool to investigate Z-DNA *in vitro* as well as *in vivo*.[4-7] Its competent ability to specifically recognize Z-conformation of nucleic acids could broaden its further uses.

Z-DNA formation is favored alternating pyrimidine-purine (APP) sequences. The sequence consisted with alternating dG and dC is formed best in Z-conformation.[8] However, other sequences of dsDNAs can also adopt Z-conformation.[8] Positively charged molecules and cations such as spermidine and cobalt hexamine can stabilize dsDNA in Z-conformation.[8] Although APP sequences are known to be ideal to form Z-DNA, out of alternating pyrimidine-purine (non-APP) sequences have been reported to form Z-DNA in a non-physiological condition.[9] The X-crystallographic study revealed that a short dsDNA with 5-methyl dCs (m5dCs) substituting dCs in d(m5CGCCm5CG/m5CGGGm5CG) could adopt Z-conformation when crystallized with magnesium and spermidine.[10] Other biophysical studies have also shown that non-APP sequences can be accommodated by the left-handed structures in non-physiological conditions.[10-12]

Ability to form Z-conformation in various DNA sequences has been assessed by Ho group using thermodynamic approaches.[13] Based on the B-Z transition energy of all possible base-pairings determined by experimentally, they developed the computer program named as `ZHUNT` that can predict relative propensity for given DNA sequences to form Z-DNA; *i.e.*, the higher Z-score means the easier to form Z-DNA in a given DNA sequence. It has been known that $Z\alpha_{ADAR1}$ is a very strong Z-forming ligand to facilitate Z-DNA formation by binding and stabilizing double-stranded nucleic acids composed of APP sequences.[14-18] Although APP sequences including d(CG) repeats and d(CA/TG) were subjects of studies for $Z\alpha_{ADAR1}$/Z-DNA interaction, the formation of Z-DNA is not restricted only to APP sequences.[19]

Our approach to study the $Z\alpha_{ADAR1}$/Z-DNA interactions in diverse non-APP sequences of dsDNAs was based on Z-score as a guidance of Z-forming potential.[13,19] DNA sequences for this study were chosen from a randomly selected region of the human genome (Accession number, AC113361.3). Their Z-scores determined by the ZHUNT program (available at http://oregonstate.edu/dept/biochem/faculty/ho.html) are summarized in Table 1. In the physiological condition with low salt concentration (10 mM HEPES, pH 7.4, 10 mM NaCl), circular dichroism (CD) was employed to monitor B-Z conversion of dsDNA oligomers in the presence of $Z\alpha_{ADAR1}$. After 30 min incubation, CD spectrum was taken and compared with that of DNA alone, *i.e.*, B-conformation of DNA. The results shown in Figure 1 demonstrated that $Z\alpha_{ADAR1}$ facilitated the conversion of dsDNA oligomers with high Z-score sequences (S1-S4)

**Table 1**. Sequences of dsDNA substrates used for B-Z conversion in this study

| Name | Sequence | Z-score |
|---|---|---|
| S1 | 5'-GGACGCCCGGGCGCCCGC-3'<br>3'-CCTGCGGGCCCGCGGGCG-5' | 2050 |
| S2 | 5'-CGGTGCGGCGCCC-3'<br>3'-GCCACGCCGCGGG-5' | 1151 |
| S3 | 5'-GGCGCGCATGGCTGGGCGCGGG-3'<br>3'-CCGCGCGTACCGACCCGCGCCC-5' | 1137 |
| S4 | 5'-CGCGCGCCGCCCGGGC-3'<br>3'-GCGCGCGGCGGGCCCG-5' | 705.4 |
| SC | 5'-AGACTCCCCAAGGCTGGG-3'<br>3'-CCCAGCCTTGGGGAGTCT-5' | 0.444 |
| CGCCCG | 5'-CGCCCG-3'<br>3'-GCGGGC-5' | n/a |
| CG3 | 5'-CGCGCG-3'<br>3'-GCGCGC-5' | n/a |

The d(CGCCCG/CGGGCG) sequence motifs are indicated by underlines. Z-scores of CGCCCG and CG3 were not determined because length of DNA must be at least 12 base pairs for calculation.

[a]This work has been carried out at the Chung-Ang University and partly at Sungkyunkwan University.

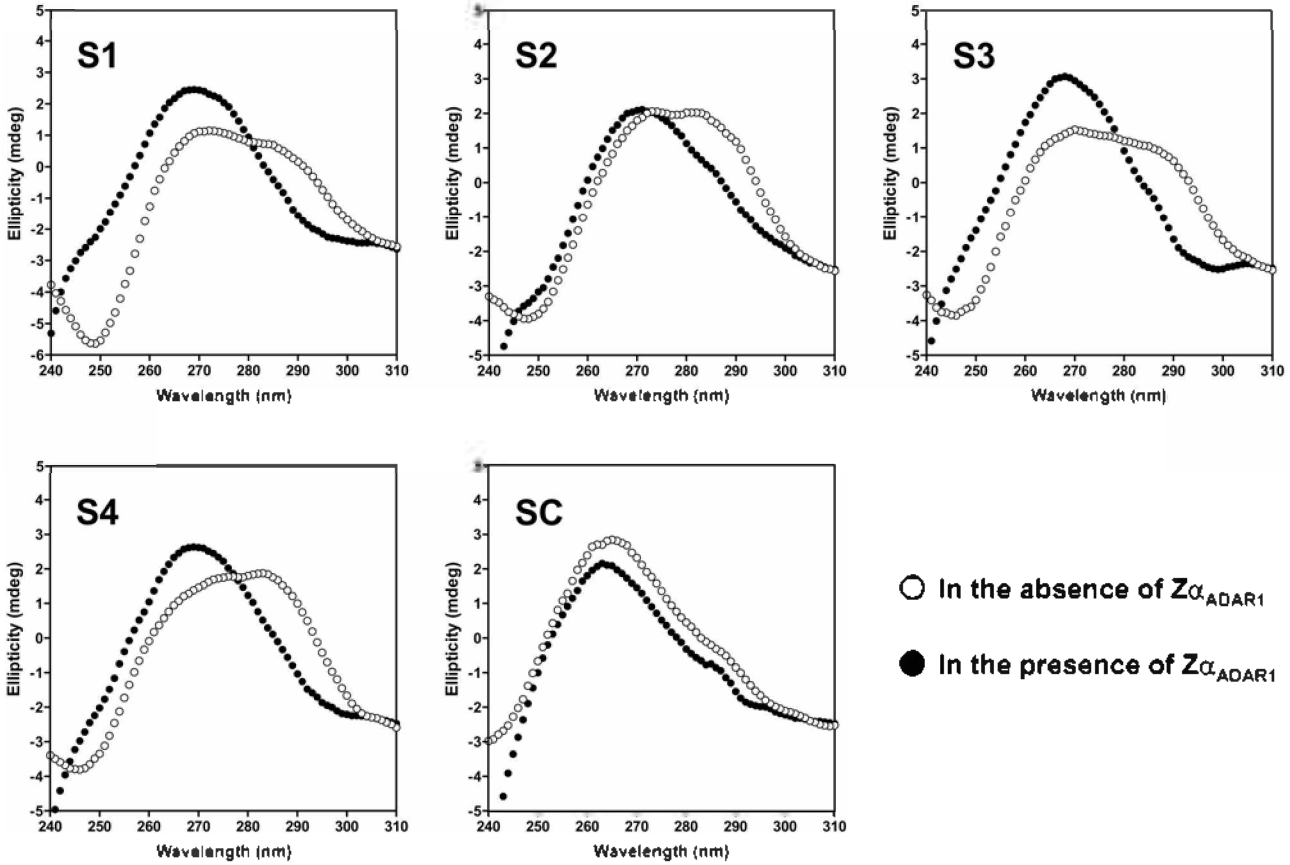


**Figure 1**. B-Z conversion of the various dsDNA substrates. CD spectra of reactions with different DNA substrates, S1-S4 and SC, at 25 °C were obtained before adding $Z\alpha_{ADAR1}$ (open circle) and in the presence of $Z\alpha_{ADAR1}$ (closed circle) after 30 min incubation, respectively. Spectral changes toward Z-DNA in high Z-score DNA samples (S1-S4) were observed after $Z\alpha_{ADAR1}$ was added, while the negative control, SC, did not show Z-DNA formation in the presence of $Z\alpha_{ADAR1}$. Inversion of the CD spectrum around 255 nm and 292 nm is a typical characteristic of B-Z conversion.

toward Z-conformation, while the negative control dsDNA (SC) with very low Z-score showed no significant Z-DNA formation in the same condition. In each DNA substrate, changes of CD spectra in B- and Z-conformations were somewhat different due to the different base compositions. However, all high Z-score dsDNAs showed typical inversed spectral changes between 250 nm and 300 nm - increase at 255 nm and decrease at 292 nm when the B to Z conversion occurs -, which is an indicative of B-Z conversion in DNA. Overall, our result demonstrated that $Z\alpha_{ADAR1}$ can also work as a powerful agent to generate B-Z conversion in a variety of sequences containing non-APP sequences.

From the study of B-Z conversion of non-APP DNAs induced by $Z\alpha_{ADAR1}$, we found that the d(CGCCCG/CGGGCG) sequence motif was presented twice in S1, and once in S4 (underlined in Table 1), respectively. The Z-DNA structure of this non-APP DNA sequence containing 5mdCs substituting dCs was previously solved by X-ray crystal diffraction analysis.[10] However, base modifications and positively charged ions used for the crystallization of d(CGCCCG/CGGGCG) was a major driving force to stabilize this non-APP sequence into Z-conformation. Thus this study is unlikely to represent that d(CGCCCG/CGGGCG) can form Z-DNA in physiological condition.

In our study, we used unmodified d(CGCCCG/CGGGCG) to test whether $Z\alpha_{ADAR1}$ can convert it into Z-DNA in physiological condition or not. The CD experiments for d(CGCCCG/CGGGCG) together with $d(CGCGCG)_2$ as a positive control were carried out at 10 °C because of their low melting temperature, 24 °C for both.[20] In Figure 2A, the CD spectra clearly showed that $Z\alpha_{ADAR1}$ can induce Z-conformation of d(CGCCCG/CGGGCG). In addition, the rates of B-Z conversion for both DNAs were also monitored (Fig. 2B). B to Z conversion of d(CGCCCG/CGGGCG) by $Z\alpha_{ADAR1}$ was faster than that of $d(CGCGCG)_2$. This result was unexpected since thermodynamic stability of $d(CGCG-CG)_2$ in Z-conformation is more stable than that of non-APP d(CGCCCG/CGGGCG). In fact, similar observation was previously reported when kinetics of B to Z conversion of various APP sequences by $Z\alpha_{ADAR1}$ was investigated.[17] Again, our result suggests that the B to Z conversion by $Z\alpha_{ADAR1}$ may require a path depending on the kinetic aspect of DNA structural change.

In summary, we examined Z-DNA formation in diverse DNA sequences in the presence of $Z\alpha_{ADAR1}$. Our result clearly demonstrated that $Z\alpha_{ADAR1}$ is capable of inducing B to Z conversion of non-APP sequences, as well as APP sequences. In the previous studies, non-APP sequences only

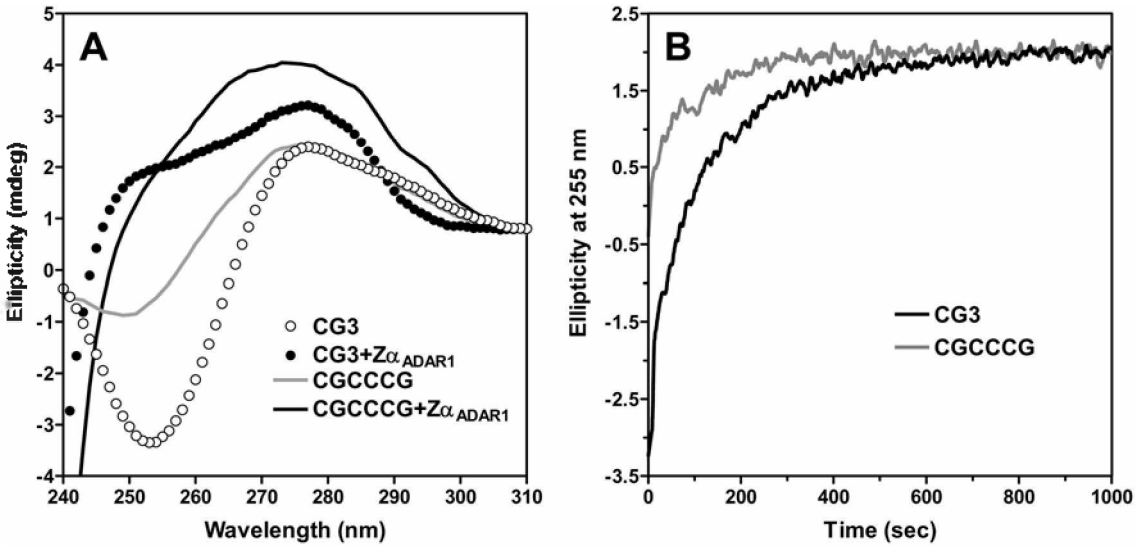


**Figure 2**. B-Z conversion and kinetic behavior of short dsDNA substrates induced by $Z\alpha_{ADAR1}$. (A) CD spectral changes of short dsDNA substrates, CGCCCG (lines) and CG3 (circles), at 10 °C were obtained before adding $Z\alpha_{ADAR1}$ (grey line or open circle) and in the presence of $Z\alpha_{ADAR1}$ (solid line or closed circle) after 30 min incubation, respectively. (B) Time-dependent change at 255 nm in the CD spectrum was monitored to determine rates of B-Z conversion of CG3 (solid line) and CGCCCG (grey line). The result showed that the B-Z conversion of CGCCCG is faster than CG3, which indicates that $Z\alpha_{ADAR1}$-induced B-Z conversion is not dependent on thermodynamic stability of DNA in Z-conformation.

formed in high salt concentration and with modified bases such as m5dCs or Br5dCs. Our work here would be a proof for the potent ability of $Z\alpha_{ADAR1}$ that facilitates Z-DNA formation in various DNA sequences. Thus, the ability of $Z\alpha_{ADAR1}$ for inducing Z-conformation of a short dsDNA containing unmodified non-APP sequence is potentially useful for studies in physiological conditions. For instance, structural Study of non-APP sequences would be accelerated by using $Z\alpha_{ADAR1}$ in physiological conditions rather than by using modified bases and high salt concentration. In addition, $Z\alpha_{ADAR1}$ may become a powerful agent to facilitate Z-DNA formation in DNA nanostructures in the future.

## Experimental Procedures

**Protein purification and DNA preparation.** The $Z\alpha_{ADAR1}$ domain (aa 133-aa 199) from hADAR1 was expressed and purified according to the previously described method with modification.[21] Briefly, the coding region of the $Z\alpha_{ADAR1}$ domain from hADAR1 cloned in pET28a vector (Novagen) was expressed with the N-terminal His-tag in *E. coli* and purified by affinity purification using a metal chelation column (Amersham Biosciences). Thrombin digestion was then carried out to remove the N-terminal His-tag. The $Z\alpha_{ADAR1}$ protein was further purified to homogeneity with a Hi-Trap SP column (Amersham Biosciences). Finally the purified $Z\alpha_{ADAR1}$ protein was dialyzed against buffer A (5 mM HEPES, pH 7.5, 10 mM NaCl), and concentrated to above 1 mM.

DNA oligonucleotides were synthesized and purified by HPLC. DNAs were dissolved in buffer B (10 mM Tris-Cl, pH 7.4, 50 mM NaCl, 0.1 mM EDTA). Annealing for dsDNA substrates was carried out by mixing each equalmolar DNA oligonucleotide to 200 $\mu$g/mL in CD buffer (10 mM HEPES, pH 7.4, 10 mM NaCl, 0.1 mM EDTA), heating for 5 min at 95 °C, and then cooling slowly to 25 °C. B-Z conversion of the dsDNA samples from each set of DNA oligonuclotides induced by $Z\alpha_{ADAR1}$ were monitored by CD (Fig. 1).

**CD measurement.** All measurements were taken on 40 $\mu$g/mL of DNA (60 $\mu$M in base pair) in CD buffer in a 0.2 cm quartz cell. CD spectra were taken at 25 °C using an Jasco J810 CD spectrometer with the exception of the short DNA substrates (10 °C for CGCCCG and CG3). Proteins were added to the sample in the final concentration of 30 $\mu$M from a concentrated stock solution, not exceeding 5% of the total volume. After 30 min incubation, CD spectra of the reaction mixtures were then recorded between 240 nm to 310 nm at 1 nm intervals averaged over 3 sec. The CD spectrum of the each dsDNAs incubated with $Z\alpha_{ADAR1}$ showed a typical Z-conformation of DNA, which confirmed that $Z\alpha_{ADAR1}$ was capable of flipping various DNA sequences containing non-APP sequences into the Z-conformation. Time-dependent change of CD spectrum at 255 nm was recorded at 1 sec intervals for 30 min, to measure the rate of B to Z conversion induced by $Z\alpha_{ADAR1}$.

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