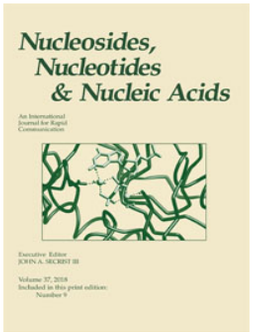

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5-Methylcytosine containing CG decamer as Z-DNA embedded sequence for a potential Z-DNA binding protein probe

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ABSTRACT

Attempting to elucidate biological significance of the left-handed Z-DNA is a research challenge due to Z-DNA potential role in many diseases. Discovery of Z-DNA binding proteins has ignited the interest in search for Z-DNA functions. Biosensor with Z-DNA forming probe can be useful to study the interaction between Z-DNA conformation and Z-DNA binding proteins. In this study, 5-methylcytosine (^{m}C) containing CG decamers were characterized for their suitability to form Z-DNA and to be used in Z-DNA forming probe. The 5′-thiol oligonucleotide embedded with 5′-^{m}CG^{m}CG^{m}CG^{m}CG^{m}CG-3′ was designed and developed as a potential Z-DNA forming probe for Z-DNA binding protein screening.

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Z-DNA; 5-methylcytosine; NMR; circular dichroism; Z-DNA binding protein

Introduction

The left handed Z-DNA was discovered in 1979 by Alexander Rich and his colleagues.[1] Although the structure of the Z-DNA is well characterized, its biological activities remain unclear. Studies on Z-DNA are now focused on its potential biological function related to the discoveries of Z-DNA binding proteins (ZBP). Thus far, a few ZBP have been identified including adenosine deaminase acting on RNA (ADAR-1),[2–4] DNA-dependent activator of IFN-regulatory factors (DAI),[5–7] and vaccinia virus E3L protein.[8–10] The role of Z-DNA in living system is believed to mediate through interaction of Z-DNA and its binding proteins. Therefore, discovery of the Z-DNA binding proteins may be the key to understand the role of Z-DNA in biological system and may provide information that can be useful as a foundation for novel drug target discovery. The objective of our research is to develop oligonucleotides based biosensor that can form

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Z-DNA which would be a very useful screening platform to search for specific ZBP. Although it has been generally known that pyrimidine-purine sequences such as CG repeated tracks are prone to form Z-DNA, promoting factors such as high salt,[11] polyamines,[12,13] or base modifications[14–17] are required to facilitate B to Z conversion. A common epigenetic modification, 5-methylcytosine (Figure 1), has been reported to promote Z-DNA formation.[18–20] Therefore, 5-methylcytosine modified CG decamer is used to construct ZBP screening probe. Structure characterization of 5-methylcytosine containing CG decamer is reported in this study. 5′-thiol modified oligonucleotides embedded with the methylated CG decamer was designed and characterized to ensure the probe capability to form Z-DNA and to bind to ZBP.

Experimental

Materials and apparatus

All oligonucleotides used in this study (Table 1) were purchased from Hokkaido System Sciences Co., Ltd. (Japan). Sheep polyclonal anti-Z-DNA antibody (Z-ab) was obtained from Abcam Ltd. (United Kingdom). Bovine serum albumin (BSA) was obtained from PAA Laboratories GmbH (USA). Analytical grade chemicals were purchased from Merck (Germany). Jasco J-815 CD spectropolarimeter with CDF-426L/15 temperature controller was used to study B to Z-DNA transition and binding between DNA and Z-ab. Bruker 600 MHz NMR spectrometer (Avance600) was used to elucidate B-DNA and Z-DNA conformation of the studied oligonucleotides.

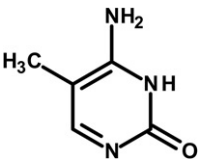

Figure 1. Chemical structure of 5-methylcytosine.

Table 1. List of the oligonucleotides used in this study.

Oligonucleotides	Base sequences	No. of base
$(CG)_5$	5′-CGC GCG CGC G-3′	10
$(^{m}C^{5}G)_5$	5′-CGC G^{m}CG CGC G-3′	10
$(^{m}CG)_5$	5′-^{m}CG^{m}C G^{m}CG ^{m}CG^{m}C G-3′	10
ODN-1	5′-R-S-S-$(CH_2)_6$- TCA AC^{m}C G^{m}CG ^{m}CG^{m}C G^{m}CG TCA AC-3′	20
ODN-2	5′-GTT GAC GCG CGC GCG GTT GA-3′	20
ODN-3	5′-R-S-S-$(CH_2)_6$- TCA ACC GCG CGC GCG TCA AC-3′	20
ODN-4	5′-R-S-S-$(CH_2)_6$- TCA ACC TAA CCA GTA TCA AC-3′	20
ODN-5	5′-GTT GAT ACT GGT TAG GTT GA-3′	20

Note: ^{m}C represents 5-methylcytosine.

B to Z-DNA transition study of (^{m}CG)$_5$ by circular dichroism

Due to previous study on modification of CG decamer $(CG)_5$,[17] the pyrimidine purine alternated sequence was continued to be used in this study. Z-DNA stabilization effect of 5-methylcytosine have been shown in various DNA sequences including poly(G^{m}C)poly(G^{m}C),[18] ^{m}CG hexamer,[19,21] and ^{m}CG tetramer,[20] but have yet to be done on CG decamer. Extrapolation of data from ^{m}CG hexamer and ^{m}CG tetramer to ^{m}CG decamer was not straightforward due to the difference in condition to obtain Z-DNA in the previous studies which used $MgCl_2$ and ethanol to promote Z-DNA formation. Therefore, in order to evaluate the methylated oligo as a potential probe for ZBP, the effect of a single (5′-CGCG^{m}CGCGCG-3′, (^{m}C^{5}G)$_5$) and multiple (5′-^{m}CG^{m}CG^{m}CG^{m}CG^{m}CG-3′, (^{m}CG)$_5$) cytosine methylation on $(CG)_5$ were investigated in this study. Circular dichroism (CD) was used to observed transition of B-DNA to Z-DNA for each oligos. The CD signal of B-DNA shows positive ellipticity at ∼280 and 220 nm and a negative ellipticity at ∼250 nm. In contrast, the CD spectrum of Z-DNA shows negative ellipticity at ∼295 nm and a positive ellipticity at ∼250 nm. The oligonucleotide samples (30 µM) were prepared in 50 mM phosphate buffer pH 7.4 with various salt concentrations range from 50 to 4000 mM NaCl. Sample cells with 1 mm pathlength was used in this experiment. The CD spectra of oligonucleotide samples were recorded from 230 nm to 310 nm at 298 K with 5 accumulations and a scanning rate of 50 nm/min.

NMR characterization of B-DNA and Z-DNA of (^{m}CG)$_5$

In complementary with CD experiment, nuclear magnetic resonance (NMR) study was conducted on $(CG)_5$ and (^{m}CG)$_5$ with Bruker 600 MHz NMR spectrometer. ^{1}H-NMR, nuclear Overhauser effect spectroscopy (NOESY), and homonuclear Hartmann–Hahn spectroscopy (HOHAHA) spectra were obtained from 100 µM oligo sample in 20 mM phosphate buffer pH 6.5 and 5% D_2O. Samples with different NaCl concentration, 50, 500, 1000, and 1500 mM, were measured to observed transition of B-DNA to Z-DNA at 283 K. All NOESY spectra were recorded with a mixing time of 300 ms. In the case of 0, 500 and 1000 mM NaCl, NOESY spectra were recorded using the 3-9-19 pulse for water suppression. In the case of 1500 mM NaCl, NOESY spectrum were recorded using the jump-and-return scheme for water suppression. The length of 90° pulses were 10, 14.5, 16.2, 43.75 µs, for samples with 0, 500, 1000 and 1500 mM of NaCl respectively. Sequential proton assignment of (^{m}CG)$_5$ in B and Z-DNA conformations was done with combined data from NOESY and HOHAHA. NMR data were processed using the software Topspin 3.5 (Bruker Biospin).

Z-DNA forming probe design and selection

The Z-DNA forming probes were designed to have four major parts (Figure 2), including 5′-alkanethiol for gold surface immobilization, Z-DNA prone $(CG)_5$ track that can convert to Z-conformation, two random non-self-complementary sequences bracing a repeated CG track to minimize non-complementary hybridization of oligonucleotides, and a complementary sequence to form DNA duplex. The negative control sequences have also been designed by replacing CG track with random sequence, which in turn diminishes the capability to form Z-DNA (Table 1). The hybridization potential of oligonucleotides were estimated with OligoCalc[22] and OligoAnalyzer Tool (Integrated DNA Technologies, Inc.) to prevent selection of sequences with high degree of self-hybridization and non-complimentary hybridization.

ODN-1 and ODN-2 duplex contains $(^{m}CG)_5{\cdot}(CG)_5$ track was expected to form Z-DNA with better degree than the rest. Although only ODN-1 contain 5-methylcytosine, the effect of the modification on one strand should prove to be sufficient to promote Z-DNA formation as seen in hairpin $(CG)_5T_4(CG)_5$ containing single C8-arylguanine modification.[23] The duplex of ODN-2 and ODN-3 was designed to have CG track and possible to form Z-DNA, while the duplex of ODN-4 and ODN-5 are complimentary and their duplex lack of CG track, hence very unlikely to form Z-DNA and was used as a negative control.

Z-DNA formation and Z-ab interaction of the designed Z-DNA forming probe

CD spectra of ODN-1 + 2, ODN-2 + 3, and ODN-4 + 5 duplexes were recorded to observe the transition of B to Z-DNA in each sequence. The oligonucleotide samples (6 μM) were annealed and prepared in 50 mM phosphate buffer pH 7.4 with various salt concentrations (50–4000 mM

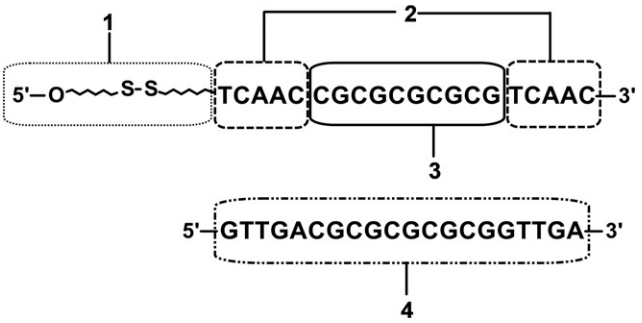

Figure 2. Structure of the designed potential Z-DNA forming probe containing (1) 5′-alkanethiol residue, (2) non-self-complementary sequences, (3) Z-DNA prone $(CG)_5$ track, and (4) a complementary oligonucleotide sequence.

NaCl) and were contained in sample cells with 10 mm pathlength. The CD spectra of oligonucleotide samples were recorded from 220 to 320 nm at 298 K with five accumulations and a scanning rate of 50 nm/min.

To study Z-ab binding to the designed probe, CD spectra of the duplex of ODN-1 + 2 or ODN-2 + 3 were obtained in the presence of Z-ab. The 3 μM duplex samples were prepared in 50 mM phosphate buffer pH 7.4. Solution of Z-ab (14 μM) in phosphate-buffered saline was purchased from Abcam. BSA (40 μM) was prepared in 50 mM phosphate buffer pH 7.4 and used as a negative control protein. Each protein was added into the oligonucleotide solution, while the binding between DNA duplex and protein was monitored by CD. The CD spectra of oligonucleotide samples were recorded from 220 to 320 nm at 298 K with a scan speed of 20 nm/min, 10 scan accumulation. Change in CD spectrum to the pattern of Z-DNA was expected to see when Z-ab was added to ODN-1 + 2, while BSA addition should produce no change.

Results and discussion

B to Z-DNA transition study of (^{m}CG)$_5$ by circular dichroism

In this study, we demonstrated that 5-methylation on C5 of $(CG)_5$ can promote B to Z-DNA transition. CD result (Figure 3) has shown that salt concentration at 2000 mM was sufficient to facilitate Z-DNA formation of $(^{m}C^{5}G)_5$ compared to 4000 mM NaCl required for unmodified $(CG)_5$ to change conformation. Increase number of 5-methylcytosine reduces amount

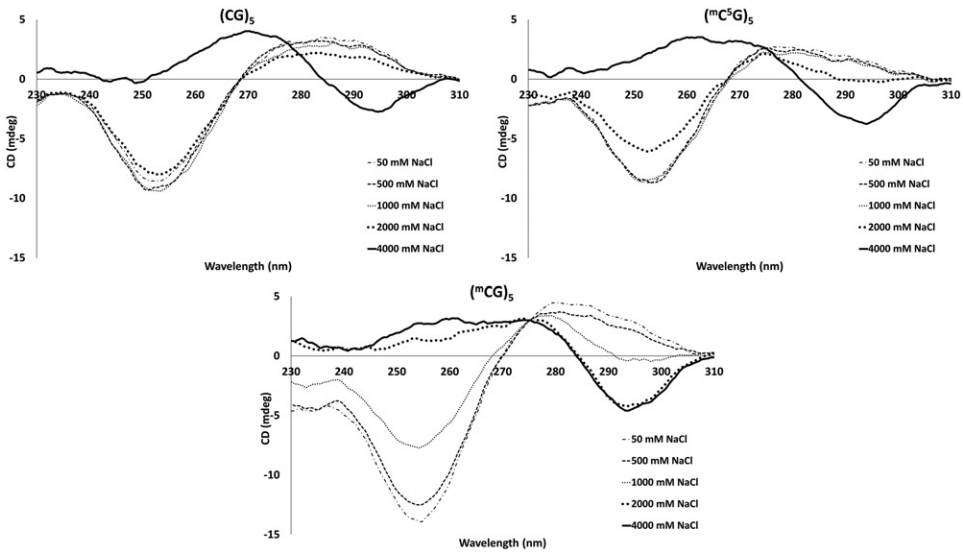

Figure 3. CD spectra of $(CG)_5$, $(^{m}C_5G)_5$ and $(^{m}CG)_5$ in various NaCl concentration. Samples were prepared in 50 mM phosphate buffer pH 7.4. Data were obtained at 298 K.

of salt to promote B to Z-DNA transition as observed from CD spectra of $(^{m}CG)_5$ which all cytosine were methylated. Amount of NaCl required to promote Z-DNA formation of $(^{m}CG)_5$ is rendered to 1000 mM as shown in Figure 3, aligned with previous study on poly($G^{m}C$)poly($G^{m}C$) that indicated the midpoint of B to Z-DNA transition is about 700 mM NaCl (5 mM Tris-HCI, pH 8.0).[18] The imbalance in middle spectra of B-Z transition of $(^{m}CG)_5$ indicates incomplete transition to Z-DNA as CD measurement for each sample was about 10 minutes, well below time required for relatively long kinetic process of B-Z transition to complete.[24] The formation of $(^{m}CG)_5$ in Z form was further studied by NMR spectroscopy.

NMR characterization of B-DNA and Z-DNA of $(^{m}CG)_5$

To confirm B to Z transition of $(^{m}CG)_5$ observed in CD study, NMR experiment was performed with samples containing 50, 500, 1000 and 1500 mM NaCl. NMR spectra of $(^{m}CG)_5$ in higher salt concentration were unable to obtained due to a limitation of the spectrometer. B to Z-DNA transition of $(CG)_5$ was not observed in all conditions in this study as expected from unmodified oligonucleotide. In the presence of low salt at 50 mM NaCl, $(^{m}CG)_5$ fully adopted B conformation. Increase in salt concentration results in B to Z-DNA transition which was observed from strong intra-residual NOE correlation between G-H8 and G-H1′ due to *syn*-conformation of the purine nucleotides[25] in Z-DNA. From G^{2}-H8 and C^{1}-H6 peaks of $(^{m}CG)_5$ of B form (Figure 4), B:Z ratio were estimated to be 3:1 in the presence of 500 mM NaCl and 1:1 in sample with 1000 mM NaCl. Furthermore, $(^{m}CG)_5$ was fully converted to Z form in 1500 mM NaCl. The intra-residual strong NOE of G-H8/G-H1′ correlation from Z-DNA was observed even in much lower amount of Z-DNA in 500 mM NaCl sample (Figure 5). It is noted that no inter-residual NOEs between H8 of G and H1′ of C were observed for 1500 mM NaCl sample, which is also expected from G residues in Z-form. The result from NMR study is correlated well with CD experiment and has confirmed the capability of $(^{m}CG)_5$ to form Z-DNA. Methylation of cytosine has been shown to drive equilibrium toward Z-DNA based on transition concentration of $(^{m}CG)_5$ which required 1000 mM NaCl compared to previously reported data of unmodified $(CG)_5$ which required 2544 mM NaCl[26] to have B:Z ratio at 1:1.

Z-DNA forming probe design and selection

The oligonucleotide probe for ZBP screening was designed to have 20 nucleotide bases in length. The principal of probe design is generally based

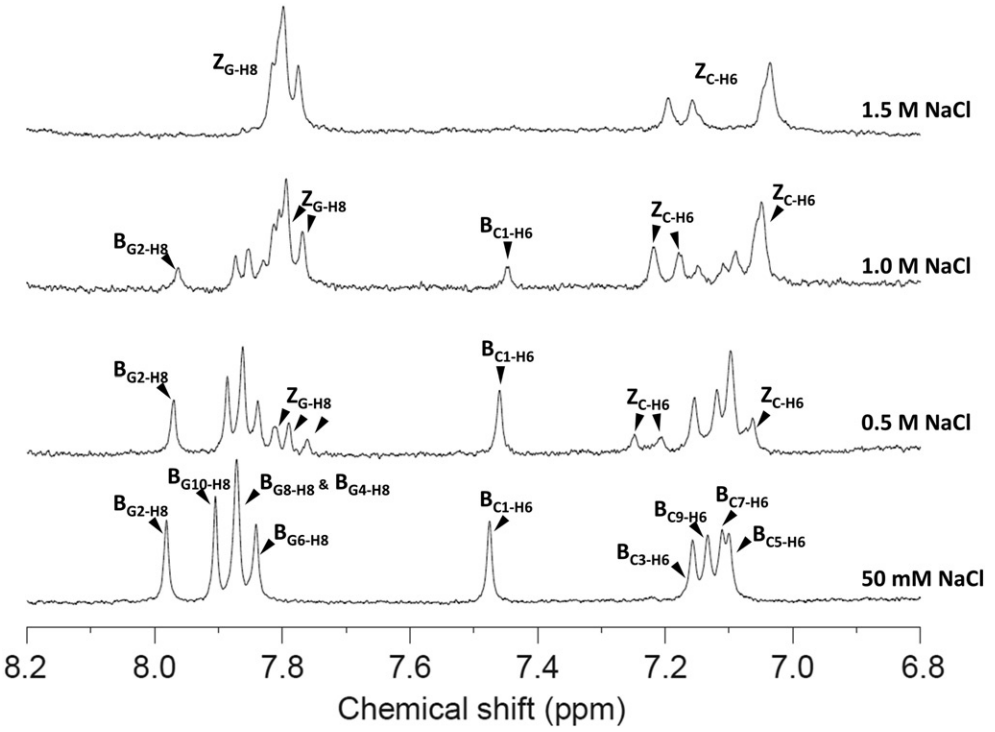

Figure 4. 1H-NMR spectra of $(^{m}CG)_5$ show G-H8 and C-H6 regions. Samples were in 20 mM phosphate buffer pH 6.5 with 50 mM NaCl where $(^{m}CG)_5$ was fully in B form, 0.5 M NaCl where B:Z ratio was 3:1, 1.0 M NaCl where B:Z was equimolar, and 1.5 M NaCl where only Z form was observed.

on our previous work on CG decemers, hence embedded sequence CG decamer was used. The alternated pyrimidine-purine, CG decamer track, was added to ODN-1 and ODN-3 as part of the probe that can convert to the left handed conformation. In addition, methylation on the C5 position of cytosine was incorporated to the CG decamer track in ODN-1 to enhance Z-DNA formation as a hemimethylated CG track. A thiol-modified linkage with six carbon spacer at 5′-end was included to facilitate immobilization process and prevent partial hybridization. The flank non self-complimentary pentamer on 5′ and 3′ were placed to prevent self-hybridization from palindromic CG decamer. The longer flank sequences would be more efficient to prevent self-dimer of thiol modified CG decamer but will render capability of duplex to form Z-DNA. Therefore, non-CG track was limited to 50% which we think that it should allow Z-DNA formation of the embedded CG decamer. The ΔG value for fully hybridized duplex of ODN-2 + 3 and ODN-4 + 5 were estimated with OligoAnalyzer. The calculated ΔG for all fully hybridized duplexes were found to be much lower than their self-dimerization, hairpin structure, or partially hybridized

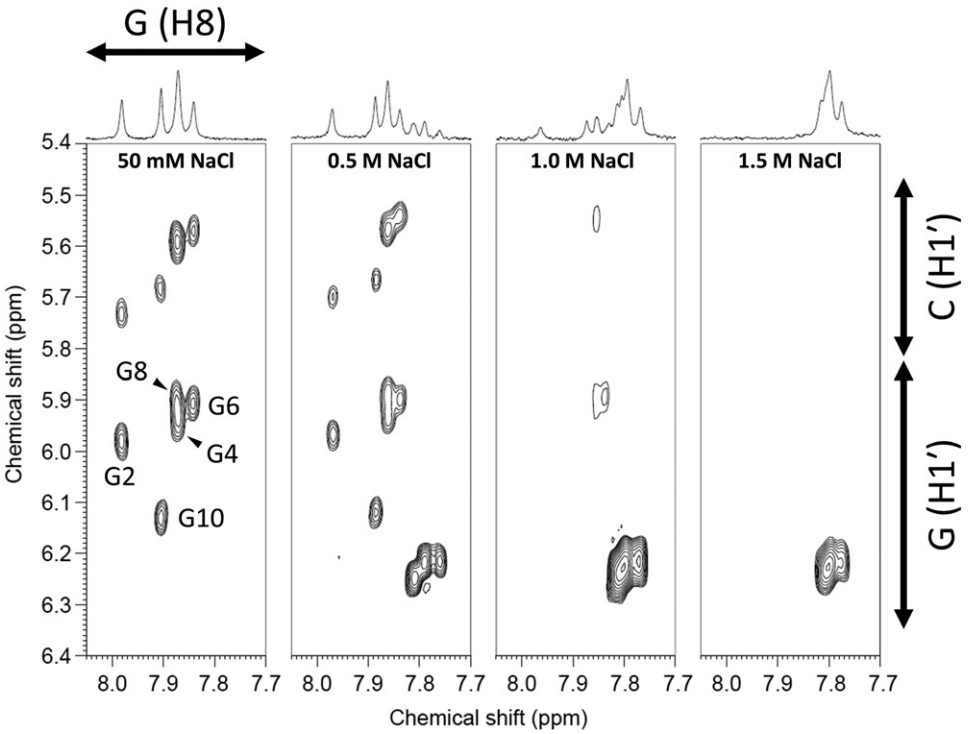

Figure 5. NOESY spectra of (^{m}CG)$_5$ demonstrate NOE correlation of G-H8/H1′. Strong NOE of syn G-H8/H1′ observed in samples with NaCl from 0.5–1.5 M indicates the presence of Z-DNA. Assignments for the intra-residual NOEs between G-H8/H1′ for B-DNA were labeled and the unlabeled NOE signals for 50 mM NaCl are due to the inter-residual NOE between G-H8/C-H1′ for B-DNA.

duplex indicate very high tendency for the selected oligonucleotides to form fully hybridization with their complimentary strands.

Z-DNA formation and Z-ab interaction of the designed Z-DNA forming probe

The key characteristic of Z-DNA forming probe is that it can convert to Z conformation. The potential of designed oligonucleotide sequences to form Z-DNA were evaluated by CD study. B-DNA and Z-DNA conformation can be distinguished by their unique CD spectra. (CG)$_5$ was known for its capability to form Z-DNA such that at low salt concentration, it adopts B-DNA conformation, while high salt concentration promotes formation of Z-DNA. The objective of the design of ODN-1 and ODN-3 to have CG decamer track in the middle of sequence is that once in duplex they should convert to the left-handed conformation in the same fashion as (CG)$_5$. According to CD study of ODN-2 + 3 (Figure 6b), the duplex was not converted to Z conformation even in high salt condition. Since Z-DNA formation is highly cooperative, it is reasonable that two B/Z-DNA junctions

from non-Z-DNA prone sequences alongside CG track may pose barrier that prevents ODN-2 + 3 to form Z-DNA which give CD result similar to the negative control duplex, ODN-4 + 5 (Figure 6c), which does not contain alternated purine-pyrimidine track and has been shown to be unable to convert to Z conformation. In contrary, the duplex of ODN-1 + 2 was expected to be much more prone to form Z-DNA based on 5-methylcytosine that facilitates Z-DNA formation. However, B- to Z-DNA conversion in CD study was also not observed for ODN-1 + 2 (Figure 6a) which represents that methylation only one side of the embedded CG duplex may not be sufficient to overcome transition barrier from B/Z-DNA junctions. Substitution of hemimethylated CG track in ODN-1 with fully methylated CG track (i.e. methylation on both ODN-1 and ODN-2) may prove to be an interesting modification to explore in future study.

Although Z-DNA formation was not present, the transition in CD spectra of ODN-1 + 2 were quite distinct from unmodified oligonucleotides. Change in negative band at 250 nm may reflect progress of local conformation change out of B-DNA. With limitation of NMR experiment at higher salt concentration, it is currently impossible to obtain high sensitivity NMR data to provide more detail on conformation of local nucleotide of ours

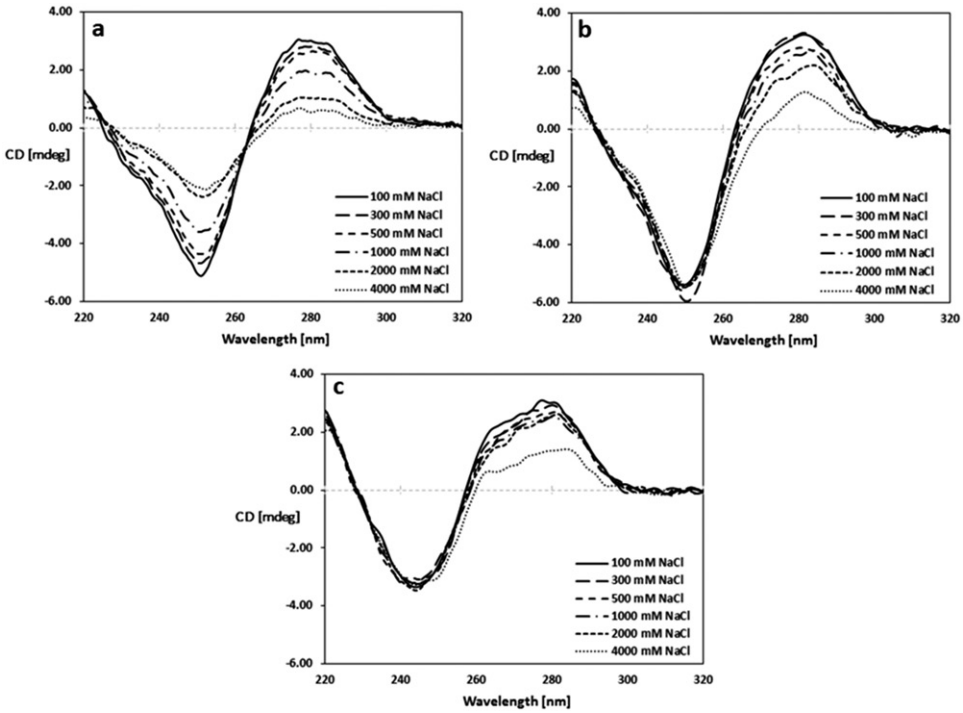

Figure 6. CD spectra of the duplexes of ODN-1 + 2 (a), ODN-2 + 3 (b) and ODN-4 + 5 (c) with NaCl range from 100 to 4000 mM.

setting. Future development on NMR condition such as the presence of a well characterized Z-DNA binding protein at low salt concentration could provide direct proof to the capability of ODN-1 + 2 to form Z-DNA.

Further study to evaluate the capability of the probe to bind to Z-ab was conducted on the 5-methycytosine containing duplex. A non Z-DNA sequence, ODN-4 + 5, was treated as a negative control DNA and it showed no change in CD when it was incubated with Z-ab (Figure 7b). In case of ODN-1 + 2, the CD spectra have shown a slight transition (Figure 7a) when Z-ab was added to the 5-methylcytosine containing oligonucleotides. From CD of ODN-1 + 2 with Z-ab, an intensity of positive ellipticity at around 280 nm gradually decreased along with changing of a negative ellipticity at ~250 nm toward positive direction when increase amount of Z-ab was added. The binding of Z-ab to ODN-1 + 2 may help stabilized Z-DNA structure, hence Z-ab facilitates the formation of Z-DNA even in relatively low Na^+ environment. Addition of Z-ab in high salt resulted in precipitation of the protein, hence unable to obtain data of Z-ab binding at high salt condition. To rule out the artifact from nonspecific DNA-protein interaction, BSA was titrate to ODN-1 + 2 duplex. Change regard to Z-DNA formation was not observed (Figure 7c) as expected from

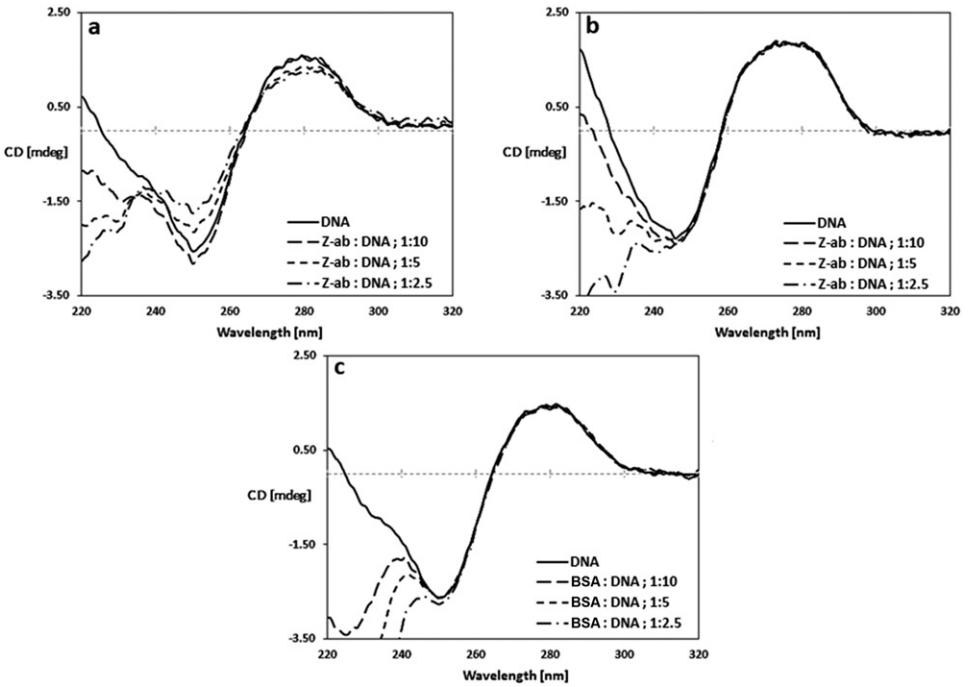

Figure 7. CD spectra of ODN-1 + 2 (a), and ODN-4 + 5 (b) in 50 mM phosphate buffer, pH 7.4 with varied molar ratio of Z-ab and oligonucleotide and CD spectra of the ODN-1 + 2 (c) in 50 mM phosphate buffer, pH 7.4 with varied molar ratio of BSA and oligonucleotide.

the negative control protein that should not bind to Z-DNA forming probe. With the results from CD, the 5-methylcytosine containing oligonucleotide has been shown to have potential for ZBP screening. Binding between different clones of Z-ab to 5-methylcytosine containing CG polymer has been shown to be diverse.[27] Depend on clone, the methyl modification may interfere with IgG interaction to Z-DNA such that binding was prevented. On the other hand, binding between Z-ab and 5-methylcytosine containing Z-DNA was observed in different clone of IgG. From superimposed image of $(^{m}CG)_5$ to $(CG)_3$ binding with DAI (PDB ID code 3EYI),[28] the methyl modifications are not in the binding region, hence they may not hindrance binding to the ZBP. Therefore, binding study on other well characterized ZBP such as ADAR-1 or DAI is the next step to ensure suitability of ODN-1 + 2 for ZBP screening. Experiment on immobilization is in progress. Immobilization of the probe and duplex formation between immobilized oligo and its complementary strand on surface plasmon resonance and quartz crystal microbalance have been conducted. Optimization of protein binding study is under investigation.

Conclusion

The methylated CG decamers have been characterized for their potential to form Z-DNA. Based on CD study and NMR elucidation of B to Z-DNA transition, $(^{m}CG)_5$ has been shown to be much more ease to convert to the left-handed conformation, which suggests its suitability for construction of ZBP screening probe. The Z-DNA forming probe was then designed to compose of 5′-thiol terminal for immobilization, Z-DNA prone hemimethylated $(^{m}CG)_5$ track, non-self complimentary track, and a complimentary strand. Z-ab binding study has suggested that the designed Z-DNA forming probe selectively binds to Z-ab which indicates its potential to be used in ZBP binding study. Nonetheless, further study is required to clearly demonstrate that the potential Z-DNA forming probe embedded with $(^{m}CG)_5$ is capable of Z-DNA formation. In addition, characterization with other known ZBP and immobilization study are underway in order to complete and to utilize the designed probe for ZBP screening.

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References

[1] Wang, A. H.; Quigley, G. J.; Kolpak, F. J.; Crawford, J. L.; van Boom, J. H.; van der Marel, G.; Rich, A. Molecular Structure of a Left-Handed Double Helical DNA Fragment at Atomic Resolution. *Nature* **1979**, *282*, 680–686.

[2] Herbert, A.; Lowenhaupt, K.; Spitzner, J.; Rich, A. Chicken Double-Stranded RNA Adenosine Deaminase Has Apparent Specificity for Z-DNA. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7550–7554.

[3] Herbert, A.; Alfken, J.; Kim, Y. G.; Mian, I. S.; Nishikura, K.; Rich, A. A Z-DNA Binding Domain Present in the Human Editing Enzyme, Double-Stranded RNA Adenosine Deaminase. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 8421–8426.

[4] Bass, B. L.; Nishikura, K.; Keller, W.; Seeburg, P. H.; Emeson, R. B.; O'Connell, M. A.; Samuel, C. E.; Herbert, A. A Standardized Nomenclature for Adenosine Deaminases That Act on RNA. *RNA* **1997**, *3*, 947–949.

[5] Takaoka, A.; Wang, Z.; Choi, M. K.; Yanai, H.; Negishi, H.; Ban, T.; Lu, Y.; Miyagishi, M.; Kodama, T.; Honda, K.; et al. DAI (DLM-1/ZBP1) Is a Cytosolic DNA Sensor and an Activator of Innate Immune Response. *Nature* **2007**, *448*, 501–505.

[6] Fu, Y.; Comella, N.; Tognazzi, K.; Brown, L. F.; Dvorak, H. F.; Kocher, O. Cloning of DLM-1, a Novel Gene That is up-Regulated in Activated Macrophages, Using RNA Differential Display. *GENE* **1999**, *240*, 157–163.

[7] Schwartz, T.; Behlke, J.; Lowenhaupt, K.; Heinemann, U.; Rich, A. Structure of the DLM-1-Z-DNA Complex Reveals a Conserved Family of Z-DNA-Binding Proteins. *Nat. Struct. Biol.* **2001**, *8*, 761–765.

[8] Liu, Y.; Wolff, K. C.; Jacobs, B. L.; Samuel, C. E. Vaccinia Virus E3L Interferon Resistance Protein Inhibits the Interferon-Induced Adenosine Deaminase A-to-I Editing Activity. *Virology* **2001**, *289*, 378–387.

[9] Kim, Y.-G.; Lowenhaupt, K.; Oh, D.-B.; Kim, K. K.; Rich, A. Evidence That Vaccinia Virulence Factor E3L Binds to Z-DNA in Vivo: Implications for Development of a Therapy for Poxvirus Infection. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 1514–1518.

[10] Kahmann, J. D.; Wecking, D. A.; Putter, V.; Lowenhaupt, K.; Kim, Y.-G.; Schmieder, P.; Oschkinat, H.; Rich, A.; Schade, M. The Solution Structure of the N-Terminal Domain of E3L Shows a Tyrosine Conformation That May Explain Its Reduced Affinity to Z-DNA in Vitro. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 2712–2717.

[11] Pohl, F. M.; Jovin, T. M. Salt-Induced Co-operative Conformational Change of a Synthetic DNA: Equilibrium and Kinetic Studies with Poly (dG-dC). *J. Mol. Biol.* **1972**, *67*, 375–396.

[12] Thomas, T. J.; Gunnia, U. B.; Thomas, T. Polyamine-Induced B-DNA to Z-DNA Conformational Transition of a Plasmid DNA with (dG-dC)n Insert. *J. Biol. Chem.* **1991**, *266*, 6137–6141.

[13] Ohishi, H.; Kunisawa, S.; van der Marel, G.; van Boom, J. H.; Rich, A.; Wang, A. H.; Tomita, K.; Hakoshima, T. Interaction Between the Left-Handed Z-DNA and

Polyamine. The Crystal Structure of the d(CG)3 and N-(2-Aminoethyl)-1,4-Diamino-Butane Complex. *FEBS Lett*. **1991**, *284*, 238–244.

[14] Sugiyama, H.; Kawai, K.; Matsunaga, A.; Fujimoto, K.; Saito, I.; Robinson, H.; Wang, A. H.-J. Synthesis, Structure and Thermodynamic Properties of 8-Methylguanine-Containing Oligonucleotides: Z-DNA under Physiological Salt Conditions. *Nucleic Acids Res*. **1996**, *24*, 1272–1278.

[15] Xu, Y.; Ikeda, R.; Sugiyama, H. 8-Methylguanosine: A Powerful Z-DNA Stabilizer. *J. Am. Chem. Soc*. **2003**, *125*, 13519–13524.

[16] Gannett, P. M.; Heavner, S.; Daft, J. R.; Shaughnessy, K. H.; Epperson, J. D.; Greenbaum, N. L. Synthesis, Properties, and NMR Studies of a C8-Phenylguanine Modified Oligonucleotide That Preferentially Adopts the Z DNA Conformation. *Chem. Res. Toxicol*. **2003**, *16*, 1385–1394.

[17] Vongsutilers, V.; Phillips, D. J.; Train, B. C.; McKelvey, G. R.; Thomsen, N. M.; Shaughnessy, K. H.; Lewis, J. P.; Gannett, P. M. The Conformational Effect of Para-Substituted C8-Arylguanine Adducts on the B/Z-DNA Equilibrium. *Biophys. Chem*. **2011**, *154*, 41–48.

[18] Behe, M.; Felsenfeld, G. Effects of Methylation on a Synthetic Polynucleotide: The B-Z Transition in Poly(dG-m5C).Poly(dG-m5dC). *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 1619–1623.

[19] Fujii, S.; Wang, A. H.; van der Marel, G.; van Boom, J. H.; Rich, A. Molecular Structure of (m5 dC-dG)3: The Role of the Methyl Group on 5-Methyl Cytosine in Stabilizing Z-DNA. *Nucleic Acids Res*. **1982**, *10*, 7879–7892.

[20] Delepierre, M.; Langlois D'Estaintot, B.; Igolen, J.; Roques, B. P. Conformational Studies of d(m5CpGpm5CpG) and d(CpGpCpG) by 1H and 31P NMR. *Eur. J. Biochem*. **1986**, *161*, 571–577.

[21] Orbons, L. P. M.; Marel, G. A.; Boom, J. H.; Altona, C. The B and Z Forms of the d(m5C-G)3 and d(br5C-G)3 Hexamers in Solution. A 300-MHz and 500-MHz Two-Dimensional NMR Study. *Eur. J. Biochem*. **1986**, *160*, 131–139.

[22] Kibbe, W. A. OligoCalc: An Online Oligonucleotide Properties Calculator. *Nucleic Acids Res*. **2007**, *35*, W43–W46.

[23] Train, B. C.; Bilgesü, S. A.; Despeaux, E. C.; Vongsutilers, V.; Gannett, P. M. Single C8-Arylguanine Modifications Render Oligonucleotides in the Z-DNA Conformation under Physiological Conditions. *Chem. Res. Toxicol*. **2014**, *27*, 1176–1186.

[24] González, V. M.; Fuertes, M. A.; Pérez, J. M.; Alonso, C. Kinetics of the Salt-Induced B- to Z-DNA Transition. *Eur. Biophys. J*. **1998**, *27*, 417–423.

[25] Orbons, L. P.; Altona, C. Conformational Analysis of the B and Z Forms of the d(m5C-G)3 and d(br5C-G)3 Hexamers in Solution. A 300-MHz and 500-MHz NMR Study. *Eur. J. Biochem*. **1986**, *160*, 141–148.

[26] Vongsutilers, V.; Gannett, P. M. C8-Guanine Modifications: Effect on Z-DNA Formation and Its Role in Cancer. *Org. Biomol. Chem*. **2018**, *16*, 2198–2209.

[27] Moller, A.; Gabriels, J. E.; Lafer, E. M.; Nordheim, A.; Rich, A.; Stollar, B. D. Monoclonal Antibodies Recognize Different Parts of Z-DNA. *J. Biol. Chem*. **1982**, *257*, 12081–12085.

[28] Ha, S. C.; Kim, D.; Hwang, H.-Y.; Rich, A.; Kim, Y.-G.; Kim, K. K. The Crystal Structure of the Second Z-DNA Binding Domain of Human DAI (ZBP1) in Complex with Z-DNA Reveals an Unusual Binding Mode to Z-DNA. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 20671–20676.