別紙様式第４

学　位　論　文　要　旨

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[論文題名]

2′-O-Methyl-8-methylguanosine as a Z-Form RNA Stabilizer for Structural and Functional Study of Z-RNA

Z 型 RNA を安定化する人工核酸 2'-O-メチル-8-メチルグアノシンの開発：Z 型 RNA の構造及び機能研究に応用

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（インパクトファクター＝3.268）

[要　旨]

The polymorphic nature of DNA can adopt the variety of conformation include A and B form and also Z- form (B form transition). Likewise, the ribonucleotide also undergoes a shift towards Z form RNA. The RNA helixes mostly adopt a right-handed A-from geometry. Another RNA conformation is characteristic of left-handed RNA containing alternating CG base pairs. *(**Nature** 1979, **282** (5740), 680-6*).

The biological role of Z-RNA is intriguing despite that after the discovery of Zα, domain from the vertebrateADAR1 enzyme. Which is bind to Z-RNA also apart from Z-DNA revealed from circulatory spectrum studies. ***(Proc Natl Acad Sci U S A 2004, 101 (6), 1514-8)***. It was strongly denoted that Z-RNA also participates some regulatory signaling pathway. Also, Zα, domain found in various protein like DLM1 and E3L both features has been involved the interferon signaling pathway initiated by identification of viral dsRNA. ***(J Mol Biol 1990, 211 (1), 147-60)***, **(*Nat Struct Biol* 2001, *8* (9), 761-5)** functionally the interferon activates the ADR1 for edit the viral genomes of persistent viruses, measles virus and hepatitis (C and D) **(*J Biol Chem* 2009, *284* (43), 29350-6)**, or are viral inhibitors of this pathway. ***(Virus Res* 1995, *36* (1), 87-96)**. Despite the collection of physical chemical and spectral data on the structural features of Z-RNA, the biological role of Z-RNA has long been in question due to the difficulty of obtaining stable Z-form oligonucleotides under physiological salt conditions.

To the study of Z-RNA stabilizer is important to understanding the biological significance of Z-RNA is essential for learning the principles of Z-RNA structures, its catalytic properties and the specificity of Z-RNA binding protein interactions. Modified or chimeric RNA studies and solution contain higher salt concentration utilized to

crystallography could be exposed the structural information about the Z-RNA. Our long term interest has synthesized a novel Z-RNA stabilizer because stabilization of Z-RNA should be intriguing for researchers because of Z form RNA stabilization required for high physiological salt concentration, also, Slow kinetics of A- to Z-RNA transition usually occur in $NaClO_4$ (6M), it was the disadvantage of fundamental analysis. The previously, reported that C8 methyl medication of guanosine dramatically stabilizes the Z form helices under physiological salt condition ***(Nucleic Acids Res.*, 1997, 25, 4589-4598).**

Several studies have been reported on incorporating modified nucleic acid residues for the stabilization of Z-DNA ***(Nucleic Acids Res.*, 1996, 24, 1272-1278).** Notably modified guanine residues highly favor the formation of Z-DNA. We have previously reported that the methylation of the C8 position of guanine significantly stabilized the Z-DNA because the modification by methylation was favorable for the *syn* conformation of the nucleobases ***(J. Am. Chem. Soc.*, 2003, 125, 13519-13524)**. Based on the results of these past studies, we have now developed a Z-form RNA stabilizer that stabilizes Z-RNA under physiological salt conditions. We designed and synthesized a 2'-*O*-methyl-8-methyl guanosine ($m^{8m}G$) by insertion of a methyl group at the C8 position of 2'-*O*-methyl guanosine. It was found that incorporation of the $m^{8m}G$ in the RNA dramatically stabilized the Z-RNA, even under physiological salt concentrations, and facilitated the A- to Z-RNA transition, even for AU-containing sequences that do not favor the formation of Z-RNA. We then determined the solution structure of $r(CGC[m^{8m}G]CG)_2$. This allowed us to see the effect of the introduction of a methyl group at the C8 position of guanosine on RNA. The Z-RNA stabilizer allowed us to understand the solution structure of Z-RNA, and it can also be used to investigate the interaction of the Zα domain and Z-RNA.

The synthesis of the $m^{8m}G$-containing oligonucleotides was carried out by phosphoramidite chemistry, beginning with 2'-O-methyl guanosine (Scheme 1). The incorporation of a methyl group in the C8 position highly favors the syn conformation. These structural features can lead the $m^{8m}G$ to greatly stabilize Z-RNA. The syn conformation of $m^{8m}G$ was experimentally confirmed by the nuclear Overhauser effect (NOE) intensity between C1′H and $8CH_3$. Circular dichroism (CD) spectroscopy is one of the more convenient methods that have been used to study the Z form conformation (***J. Am. Chem. Soc.*, 2003, 125, 13519-13524)**. A-RNA, a negative Cotton effect appears at 295 nm, **(*J. Am. Chem. Soc.*, 2011, 133, 2016-2018)** whereas in Z-RNA, a more positive intense band appears at 280 nm. Thus, we performed CD spectroscopy experiments to monitor the conformational state at various $NaClO_4$ concentrations. ***(J***

***Am Chem Soc* 2003, *125* (44), 13519-24)**.

We used the CD spectroscopy to examine the A-Z transition under various salt concentrations. Native RNA r(CGCGCG)$_2$ in A-form did not undergo a transition to the Z form at all, even in the presence of 3 M $NaClO_4$ (Fig. 1a). $m^{8m}G$-incorporated r($C_1G_2C_3[m^{8m}G]_4C_5G_6)_2$ showed a CD spectrum typical of the Z form with increasing concentrations of $NaClO_4$. (Fig. 1b). The midpoint $NaClO_4$ concentration for r($C_1G_2C_3[m^{8m}G]_4C_5G_6)_2$ was 900 mM, lower than that of the unmodified duplex (4100 mM) . r(C[m^{8m}G]C[m^{8m}G]CG)$_2$ containing two m^{8m}Gs greatly stabilized the Z form, showing a CD spectrum typical of the Z form even in the presence of 50 mM $NaClO_4$ at a lower physiological salt concentration, and the midpoint was 100 mM. Significant stabilization of the Z form by incorporation of $m^{8m}G$ was observed in a duplex containing an AU base pair, which showed the typical Z form only at 5 mM with the 2400 mM midpoint compared to the native RNA.

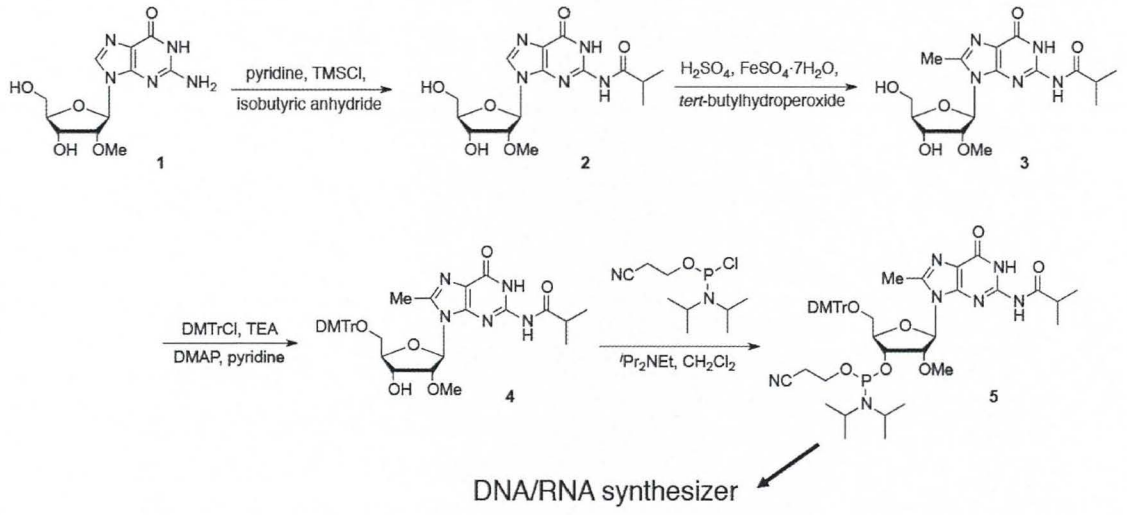
To further understand the $m^{8m}G$ effect on the thermodynamic properties of Z-RNA, the melting temperature (T_m) and thermodynamic parameters were examined using CD melting experiments. Thermodynamic parameters revealed a very favourable free energy formation for the $m^{8m}G$-incorporated Z-RNA compared to the native RNA suggesting that the syn conformation of $m^{8m}G$ was thermodynamically favorable, and the preferred C3′ endo conformation of ribose induced by the 2'-O-methyl group also contributed to the stabilization of the Z form, which is consistent with the previous study **(*Nucleic Acids Res*, 1995, 23, 2019-2024).**

To further obtain the structural features and confirm observation of Z-RNA by NMR study. We constructed a model of r($C_1G_2C_3[m^{8m}G]_4C_5G_6)_2$ based on the reported Z form structure and NOE-constrained refinement. The molecular dynamics simulations were performed by the standard dynamics cascade in BIOVIA Discovery Studio 4.5 with some modifications. The conformation with the lowest energy was selected as shown in Fig. 2. In the $m^{8m}G$-modified Z-RNA structure, the hydrophobic C8-methyl groups were located in the periphery of the helix and prominently exposed to the solvent region, which is consistent with the induction of a methyl group that strongly contributes to the increased stabilization of Z-RNA.

Encouraged by the ability to use $m^{8m}G$ to stabilize Z-RNA, we study the interaction of the Zα domain and Z-RNA by using $m^{8m}G$-containing a Z-RNA and Zα-EGFP fusion protein, in which the Zα domain is tagged with a green fluorescence protein. **(*Structure*, 2007, 15, 395-404)**, Fig. 3 indicates the visualized mode as EGFP-mode, Cy3-mode and Merge, respectively. Lane 1, lane 2 and lane 3 in Fig. 3 show free Z-RNA labeling with fluorescent dye Cy3, a complex of Z-RNA and Zα-EGFP and free

Zα-EGFP, respectively. Green and red fluorescence emission visualized under EGFP-mode and Cy3-mode distinguished Zα-EGFP and Z-RNA. The complex formed by the Z-RNA and Zα-EGFP was directly visualized in the upper portion of the gel because of the large molecular weight of Zα-EGFP. Lane 2 in merge mode of Fig. 3 emitted yellow fluorescence and clearly demonstrated the complex of Zα with Z-RNA. These observations indicated that Zα-EGFP efficiently binds to the $m^{8m}G$-containing Z-RNA without severe steric clashes with the added methyl groups, suggesting the $m^{8m}G$ stabilized Z-RNA can be used to study the interaction of Z-RNA with proteins.

In conclusion, the results described here reveal that the newly synthesized guanosine analogue 2'-*O*-methyl-8-methyl guanosine dramatically stabilizes Z-RNA, which arises from the *syn* conformation of the $m^{8m}G$ base and the C3' *endo* conformation of ribose. It allows oligonucleotides with a wide range of sequences of AU to be converted into Z-RNA. In addition, researchers can utilize the Z-RNA stabilizer to study the interaction of the Zα domain with Z-RNA sequences. Using the Z-RNA stabilizer. To the best of our knowledge, this is the first example of a Z-RNA stabilizer.



Scheme 1. Synthetic scheme of 2'-*O*-methyl-8-methylguanosine phosphoramidite **5** and $m^{8m}G$-containing RNA oligonucleotides.

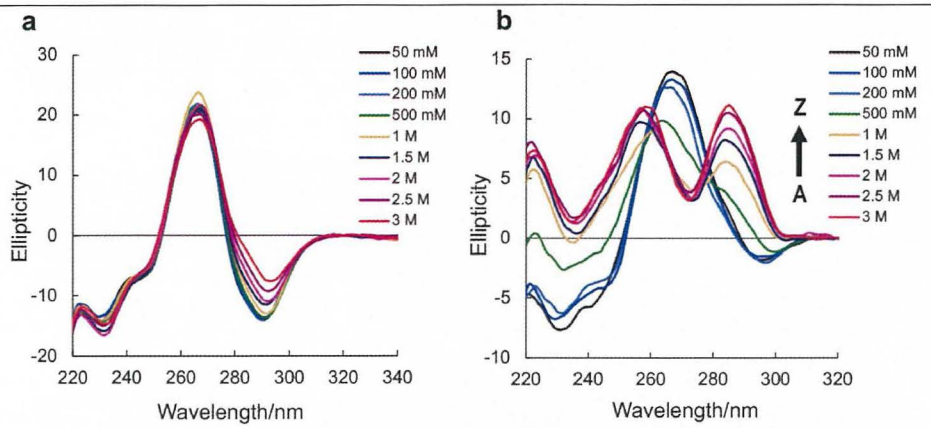


Fig. 1 CD spectra of native RNA r(CGCGCG)$_2$ (**a**) and m^{8m}G-contained r(CGC[m^{8m}G]CG)$_2$ (**b**) in 5 mM Na_2HPO_4 buffer, pH 7.0, at 10 °C at various $NaClO_4$ concentrations (0.15 mM base concentration).

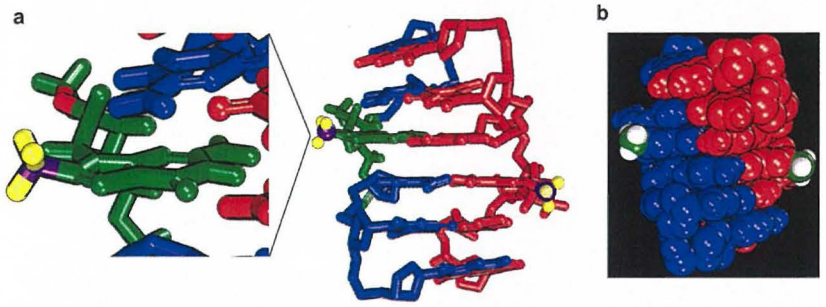


Fig. 2 The model for r(C$_1$G$_2$C$_3$[m^{8m}G]$_4$C$_5$G$_6$)$_2$ Z-RNA structure. (**a**) m^{8m}G is expanded in green with the C8-methyl group, hydrogen and carbon of methyl group are coloured in yellow and purple. (**b**) Hydrogen and carbon of methyl group are coloured in green and white. C8-methyl groups were located outside of Z-RNA.

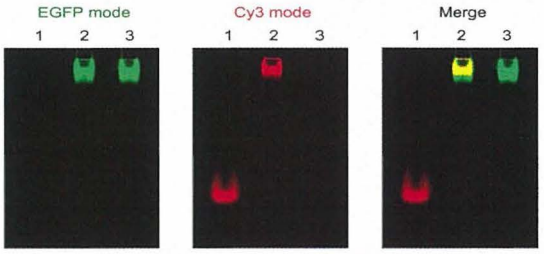


Fig. 3 Visualization of Z-RNA and Zα-EGFP protein. Lane 1: Z-RNA only, Lane 2: RNA + Zα-EGFP, Lane 3: Zα-EGFP only. The different modes are indicated in upper. [RNA] = 1 μM, [Zα-EGFP] = 10 μM, [NaCl] = 100 mM, [Tris-HCl (pH7.0)] = 10 mM, [DTT] = 5 mM, 10% glycerol, 10 μg/mL BSA.

備考　論文要旨は、和文にあっては 2，000 字程度、英文にあっては 1，200 語程度