論文の内容の要旨

Metal-responsive Bifacial DNA Base Pairing based on 5-Hydroxyuracil Nucleobase

（5-ヒドロキシウラシル核酸塩基を用いた金属応答性二面型 DNA 塩基対の開発）

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1. Introduction

DNA molecules form duplexes, triplexes, and other higher-order structures in a sequence-specific manner. Thus, DNAs have great potential to serve as building blocks of nanoarchitectures and nanomachines. We and other groups have developed artificial metallo-DNAs, which contain an unnatural nucleobase pair formed through metal coordination bonding instead of natural hydrogen-bonding.[1] The metal-mediated base pairing allows thermal stabilization of self-assembled and folded DNA structures and thereby metal-induced DNA structural conversion. Moreover, introduction of multiple metal-mediated base pairs provides the fundamentals for precise metal arrangement in the DNA helical structures.

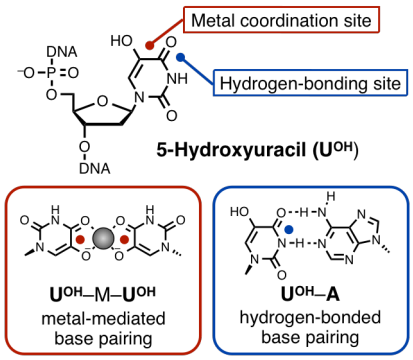

Figure 1. Metal-responsive bifacial DNA base pairing of 5-hydroxyuracil ($\mathbf{U}^{\mathbf{OH}}$) nucleobase. $\mathbf{U}^{\mathbf{OH}}$ base forms both a metal-mediated base pair ($\mathbf{U}^{\mathbf{OH}}$–M–$\mathbf{U}^{\mathbf{OH}}$) and a hydrogen-bonded base pair ($\mathbf{U}^{\mathbf{OH}}$–**A**).

In this study, I have utilized a 5-hydroxyuracil ($\mathbf{U}^{\mathbf{OH}}$) nucleobase for metal-responsive "*bifacial*" nucleobase pairing (Figure 1). The 4-carbonyl and 5-hydroxy groups of $\mathbf{U}^{\mathbf{OH}}$ nucleobase can serve as a bidentate metal ligand, in which its uracil scaffold is maintained as a hydrogen bond donor/acceptor site. Therefore, it was expected that the $\mathbf{U}^{\mathbf{OH}}$ nucleobase may form a metal-mediated base pair ($\mathbf{U}^{\mathbf{OH}}$–M–$\mathbf{U}^{\mathbf{OH}}$, M = metal ion) as well as a hydrogen-bonded base pair with adenine (**A**) nucleobase ($\mathbf{U}^{\mathbf{OH}}$–**A**), leading to stabilization effects and dynamic switching functions based on the metal-responsive *bifacial* base pairing.

2. Metal-mediated DNA base pairing of 5-hydroxyuracil nucleobases

This study aimed to construct novel metallo-DNA duplexes through the formation of metal-mediated $\mathbf{U}^{\mathbf{OH}}$–M–$\mathbf{U}^{\mathbf{OH}}$ base pairs. A DNA duplex **1**·**2**, containing three $\mathbf{U}^{\mathbf{OH}}$–$\mathbf{U}^{\mathbf{OH}}$ base pairs, was synthesized by a DNA synthesizer (Figure 2a).[2,3] The effects of metal ions on the thermal stability of DNA duplex **1**·**2** were estimated by melting experiments. The melting temperature (T_m) of the DNA duplex **1**·**2** (Figure 2b) was increased upon addition of Zn^{II} (ΔT_m = + 13 °C) or a series of lanthanide ions [e.g. Gd^{III} (ΔT_m = + 18 °C)]. The results suggest that the formation of $\mathbf{U}^{\mathbf{OH}}$–M–$\mathbf{U}^{\mathbf{OH}}$ base pairs conferred interstrand crosslinking through the metal coordination bonds. In particular, some lanthanide ions including Gd^{III} exhibited significant duplex stabilization. UV absorption-based titration experiment was then conducted for the DNA duplex **1**·**2** with Gd^{III} ions. A new UV absorption band around 310 nm gradually increased until the ratio of [Gd^{III}] to [duplex **1**·**2**] reached 3.0. In addition, ESI-TOF mass analysis proved the formation of a trinuclear Gd^{III} complex (**1**·**2**·$\mathbf{Gd}^{\mathbf{III}}_{\mathbf{3}}$) (found: 1368.77 ($z$ = 7); calcd for [**1** + **2** + 3Gd –16H]$^{7-}$: 1368.73) with three $\mathbf{U}^{\mathbf{OH}}$–Gd^{III}–$\mathbf{U}^{\mathbf{OH}}$ base pairs inside the DNA duplex **1**·**2** (Figure 2c).

Circular dichroic analysis was further conducted to clarify the DNA structure. As a result, Cotton effects of typical B-form DNA duplex were observed even in the presence of 3 equiv. of Gd^{III} ions although the intensity was decreased. This observation suggests that the Gd^{III} complex of duplex **1**·**2** has a structure similar to B-form,

which was further confirmed by NMR spectroscopy.

Taken all together, the formation of metallo-DNA duplexes containing $\mathbf{U^{OH}}$–M–$\mathbf{U^{OH}}$ (M = Zn^{II}, Gd^{III} etc.) was successfully demonstrated. This metal-mediated base pairing thermally stabilizes the resulting DNA duplexes, allowing quantitative assembly of Gd^{III} ions inside double-stranded DNA structures.

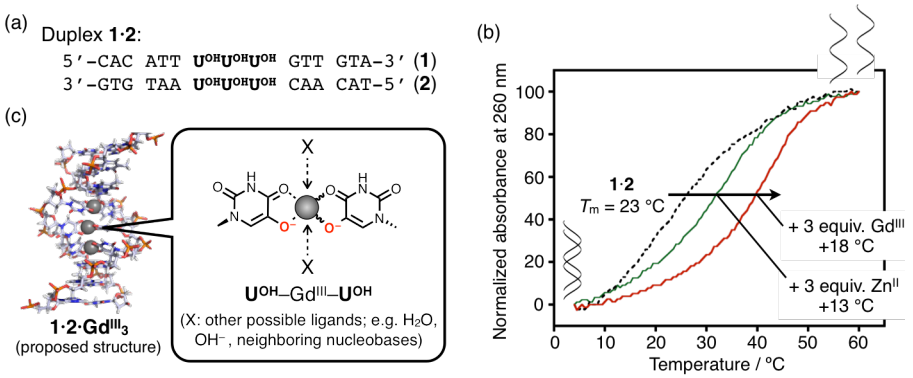

Figure 2. Construction of novel metallo-DNA duplexes based on $\mathbf{U^{OH}}$ nucleobases. (a) DNA sequences. (b) Effects of metal ions on the melting behaviors of the DNA duplex **1·2**. [duplex] = 2 μM, [$GdCl_3$]/[duplex] or [$ZnSO_4$]/[duplex] = 0 or 3 in 10 mM HEPES buffer (pH 8.0), 100 mM NaCl. (c) A proposed structure of the metallo-DNA **1·2·Gd$^{III}_3$**.

3. Metal-mediated regulation of DNA hybridization preference using 5-hydroxyuracils

A $\mathbf{U^{OH}}$ nucleobase also forms a hydrogen-bonded $\mathbf{U^{OH}}$–**A** base pair (**A** = adenine). I expected that the stability of the $\mathbf{U^{OH}}$–**A** base pair can be controlled by metal complexation. Firstly, the effect of metal ions was examined on the stability of DNA duplex **1·2A**, containing three $\mathbf{U^{OH}}$–**A** base pairs in the central region (Figure 3a). The melting temperature (T_m) of duplex **1·2A** was decreased by 14 °C upon addition of 3 equiv. of Gd^{III} (Figure 3b; **X** = **A**). In addition, its UV absorption spectral changes suggested the binding of Gd^{III} to the metal coordination site of $\mathbf{U^{OH}}$ nucleobase concurrently with deprotonation of the 5-hydroxy group of $\mathbf{U^{OH}}$. This destabilization effect was not observed with the other DNA duplexes containing three mismatched base pairs [$\mathbf{U^{OH}}$–**X**; **X** = **T** (thymine), **G** (guanine), or **C** (cytosine)] (Figure 3). These results indicate that the hydrogen bonding between $\mathbf{U^{OH}}$ and **A** nucleobases was weakened by the binding of metal ions to $\mathbf{U^{OH}}$–**A** base pairs presumably due to the electronic and steric effects. Thus, the $\mathbf{U^{OH}}$–**A**-containing DNA duplex was selectively destabilized through the Gd^{III} complexation of $\mathbf{U^{OH}}$ bases.

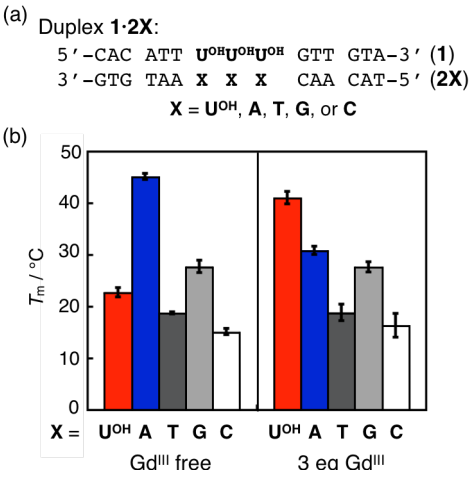

Figure 3. Hybridization preference of $\mathbf{U^{OH}}$-containing DNA strands. (a) DNA sequences. (b) Melting temperatures (T_m) of the DNA duplexes **1·2X**. [duplex] = 2 μM, [$GdCl_3$]/[duplex] = 0 or 3 in 10 mM HEPES buffer (pH 8.0), 100 mM NaCl. Error bars indicate the standard deviations.

Next, I examined the hybridization preference of the $\mathbf{U^{OH}}$-containing DNA strands. In the absence of Gd^{III} ion (Figure 3b, left), the DNA duplex **1·2A** was more stable than the duplex **1·2** due to the hydrogen-bonded $\mathbf{U^{OH}}$–**A** base pairing. In the presence of 3 equiv. of Gd^{III} ions (Figure 3b, right), the DNA duplex **1·2** was more stable because of the stabilization through the $\mathbf{U^{OH}}$–Gd^{III}–$\mathbf{U^{OH}}$ base pairing as well as the destabilization of duplex **1·2A** based on the Gd^{III} complexation of $\mathbf{U^{OH}}$–A base pairs. Thus, the hybridization preference of DNA strand containing $\mathbf{U^{OH}}$ nucleobases was significantly influenced by the Gd^{III} complexation.

In addition, I investigated pH dependence of the metal-induced stabilization and destabilization of the $\mathbf{U^{OH}}$-containing DNA duplexes. Melting experiments revealed that Zn^{II} ion affected the duplex stability in a pH-dependent manner. Upon addition of 3 equiv. of Zn^{II}, the T_m value of the DNA duplex **1·2** was increased by 13 °C and 22 °C at pH 8.0 and 9.0, respectively, while it was hardly changed at pH 7.0. Furthermore, UV absorption spectral changes indicated that the metal complexation occurred only under basic conditions. These results are well consistent with the fact that the metal complexation of $\mathbf{U^{OH}}$ nucleobases is accompanied with

deprotonation of the 5-hydroxy group, whose pK_a value is 7.7.[4] In addition, the Zn^{II} addition was found to induce thermal destabilization of duplex **1·2A** in a pH-dependent manner. Therefore, the thermal stability of $\mathbf{U^{OH}}$-containing duplex can be controlled not only by metal ions but also by pH when Zn^{II} was employed.

4. Metal-driven DNA strand exchange reactions through base pair switching of 5-hydroxyuracils

Next, I examined DNA strand exchange reactions through the metal-driven base pair switching between $\mathbf{U^{OH}}$–**A** and $\mathbf{U^{OH}}$–Gd^{III}–$\mathbf{U^{OH}}$ base pairs (Figure 4a). It was expected that the exchange reactions could take place reversibly and isothermally based on the metal-mediated stabilization and destabilization. Then, native polyacrylamide gel electrophoresis (PAGE) analysis was conducted to investigate the effects of Gd^{III} on the hybridization preference of the template DNA strand **1**. A mixture of DNAs **1**, the complementary strands **2**, and **2A** was annealed to obtain thermodynamically stable products, in which the strand **2A** was labeled with a fluorescence tag (FAM) for gel analysis. Whereas the duplex **1·2A** containing three $\mathbf{U^{OH}}$–**A** base pairs was quantitatively formed in the absence of Gd^{III}, the metallo-DNA duplex **1·2** with Gd^{III} was formed in ~40% yield in the presence of 3 equiv. of Gd^{III}. After optimization of experimental conditions, I found that another template DNA **3** containing four $\mathbf{U^{OH}}$ nucleobases and complementary DNA strands **4** and **4A** (Figure 4b, left) in the presence of 6 equiv. of Gd^{III} preferentially generated metallo-DNA **3·4** with Gd^{III} in ~80% yield. These results show that the hybridization preference can be controlled by Gd^{III} complexation based on the *bifacial* behaviors of $\mathbf{U^{OH}}$ nucleobases.

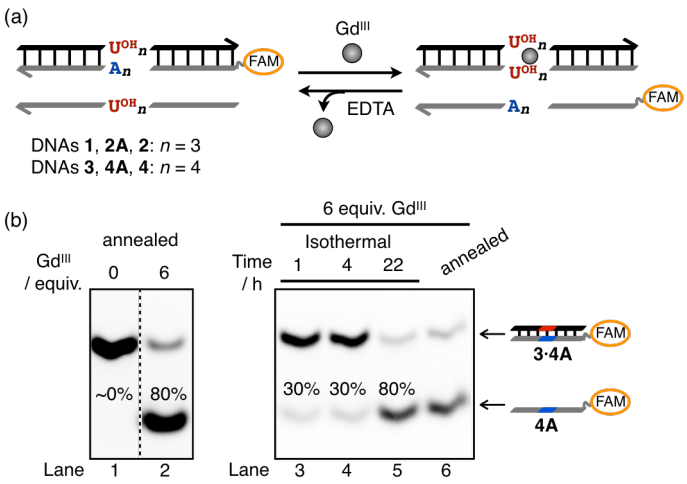

Figure 4. Metal-responsive DNA strand exchange reactions through the base pair switching of $\mathbf{U^{OH}}$ nucleobases. (a) Schematic representation of the metal-responsive strand exchange and DNA sequences. (b) Native PAGE for DNAs **3**, **4A** and **4**. [DNA] = 2 μM for each, [$GdCl_3$]/[DNA] = 0 or 6 in 10 mM HEPES buffer (pH 8.0), 100 mM NaCl. The reaction mixture was annealed after (lanes 2, 6) or before adding Gd^{III} ions (lanes 3–5). 20% acrylamide gel in TAMg buffer (pH 8.0; 40 mM Tris, 7.6 mM $MgCl_2$, 1.4 mM acetic acid), 4 °C.

I subsequently investigated whether the DNA strand exchange reactions take place under isothermal conditions, that is, without the annealing process. A mixture of DNA duplex **3·4A**, which contains four $\mathbf{U^{OH}}$–**A** base pairs, and single strand **4** was prepared in the absence of Gd^{III} ion. After addition of 6 equiv. of Gd^{III}, the reaction mixture was incubated at 30 °C for 22 h and the products were analyzed by native PAGE analysis. The result showed that the duplex **3·4A** was converted to the metallo-DNA duplex **3·4** with Gd^{III} in ~80% yield (Figure 4b, right). Furthermore, when 6 equiv. of EDTA was added to remove Gd^{III}, the reverse reaction proceeded quickly and quantitatively. The result shows that this strand exchange reaction is reversible. Consequently, the introduction of *bifacial* $\mathbf{U^{OH}}$ nucleobases into DNA allows reversible metal-responsive DNA strand exchange reactions, which may possibly serve as a motive power in DNA-based nanomachines.

5. Metal-responsive triplex-forming oligonucleotides based on 5-hydroxyuracils

Triple-stranded DNAs are generally formed based on the sequence-specific recognition of a DNA duplex with a triplex-forming oligonucleotide (TFO), that is, the formation of base triplets such as **T**•**A**–**T** and $\mathbf{C^+}$•**G**–**C** ($\mathbf{C^+}$ is protonated **C**). The binding of TFO inhibits access of proteins as shown in RNA polymerase and histone to the template DNA, which would be utilized to artificially regulate gene expression.[5] It was expected that $\mathbf{U^{OH}}$ nucleobase would form a $\mathbf{U^{OH}}$•**A**–**T** base triplet leading to a triplex structure like the canonical **T**•**A**–**T** base triplet,

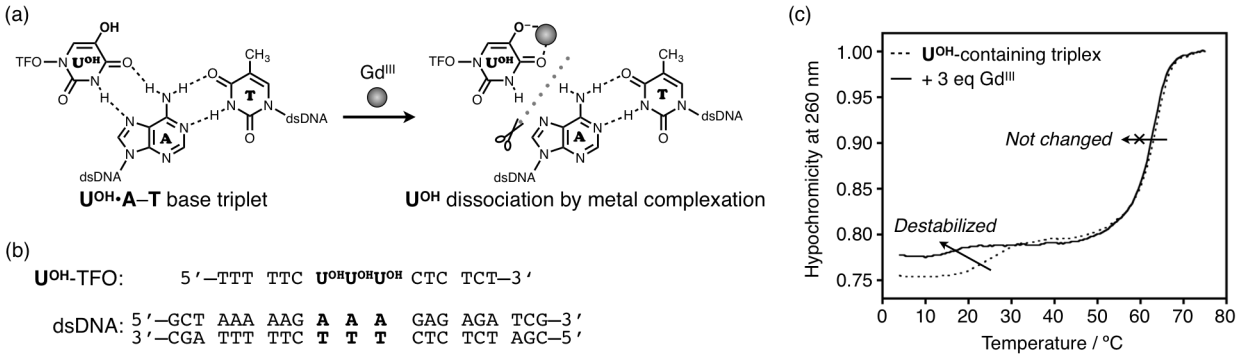

Figure 5. $\mathbf{U^{OH}}$-containing triplex-forming oligonucleotide ($\mathbf{U^{OH}}$-TFO) for metal-responsive structural conversion between triplex and duplex. (a) Metal complexation of $\mathbf{U^{OH}}$•**A**–**T** base triplet. (b) Sequences of DNA strands employed. (c) Melting curves of the $\mathbf{U^{OH}}$-containing triplex ($\mathbf{U^{OH}}$-TFO + dsDNA). [DNA] = 1.5 μM for each, [$GdCl_3$]/[triplex] = 0 or 3 in 10 mM HEPES buffer (pH 7.0), 140 mM NaCl, 10 mM $MgCl_2$, 0.2 °C/min.

and that metal complexation of the $\mathbf{U^{OH}}$•**A**–**T** base triplet may cause dissociation of TFO from the main duplex structure (Figure 5a). Thus, the $\mathbf{U^{OH}}$-containing TFO would be applied for the regulation of transcription in response to metal ions. As the first step, I investigated the thermal stability of a triple-stranded DNA based on metal complexation of $\mathbf{U^{OH}}$ nucleobases. Thermal melting behaviors of a triple-stranded DNA consisting of a $\mathbf{U^{OH}}$-containing TFO ($\mathbf{U^{OH}}$-TFO) and a target natural duplex (dsDNA) were analyzed with or without Gd^{III} ions (Figure 5b, c). The first transition was ascribable to the dissociation of $\mathbf{U^{OH}}$-TFO from dsDNA, and the second transition was due to the dissociation of dsDNA into the single strands. Upon addition of 3 equiv. of Gd^{III} ions, the triplex was thermally destabilized. In contrast, the thermal stability of dsDNA was hardly perturbed by the Gd^{III} addition. Thus, the binding affinity of the $\mathbf{U^{OH}}$-TFO to the natural DNA duplexes can be regulated in response to Gd^{III} ions. This metal-triggered dissociation of $\mathbf{U^{OH}}$-TFO would be applied for the regulation of biological functions such as transcription.

6. Conclusion

I have successfully developed metal-responsive *bifacial* DNA base pairing based on 5-hydroxyuracil ($\mathbf{U^{OH}}$) nucleobase. (i) Metal-mediated $\mathbf{U^{OH}}$–M–$\mathbf{U^{OH}}$ base pairing stabilized the DNA duplex in response to Zn^{II} and lanthanide metal ions such as Gd^{III}, whereas hydrogen-bonded $\mathbf{U^{OH}}$–**A** base pairing was destabilized by these metal ions. (ii) The thermal stability of Zn^{II}-mediated base pairs can be controlled by pH changes. (iii) The *bifacial* base pairing was applied to the metal-responsive DNA strand exchange reactions through the reversible base pair switching of $\mathbf{U^{OH}}$ nucleobases. (iv) The binding affinity of the triplex-forming DNA oligonucleotide ($\mathbf{U^{OH}}$-TFO) to dsDNA was regulated in response to Gd^{III} addition.

The metal-responsive stability controls and DNA strand exchange reactions would be applied for reversible regulation of DNA nanostructures such as DNA origami, DNA polyhedral, and logic gate. The $\mathbf{U^{OH}}$-TFO could serve as an artificial transcription factor to dynamically control gene expression. Thus, I believe that the metal-responsive *bifacial* base pairing developed in this study would greatly contribute to the development of DNA nanotechnology.

References

[1] Y. Takezawa, M. Shionoya, *Acc. Chem. Res.* **2012**, *45*, 2066. [2] J. Fujimoto, L. Tran, L. C. Sowers, *Chem. Res. Toxicol.* **1997**, *10*, 1254. [3] M. L. Morningstar, D. A. Kreutzer, J. M. Essigmann, *Chem. Res. Toxicol.* **1997**, *10*, 1345. [4] C. J. La Francois, Y. H. Jang, T. Cagin, W. A. Goddard, III, L. C. Sowers, *Chem. Res. Toxicol.* **2000**, *13*, 462. [5] B. P. Casey, P. M. Glazer, *Prog. Nucleic Acid Res. Mol. Biol.* **2001**, *67*, 163.