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Metal-responsive Reversible Binding of Triplex-forming Oligonucleotides with 5-Hydroxyuracil Nucleobases

Kotaro Nishiyama, Keita Mori, Yusuke Takezawa and Mitsuhiko Shionoya*

Department of Chemistry, Graduate School of Science, The University of Tokyo 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033 (Japan)
E-mail: shionoya@chem.s.u-tokyo.ac.jp

Contents

1. Materials and methods

DNA synthesis

DNA oligonucleotides containing 5-hydroxyuracil (**U^{OH}**) and 5-methylcytosine nucleobases were prepared according to the reported procedure.[1-3] DNA strands were synthesised on an NTS M-2-MX DNA/RNA synthesiser (Nihon Techno Service) using ultramild phosphoramidites and reagents (Glen Research). The products were deprotected using 28% NH_3 aqueous solution at room temperature for 2–3 h and then purified and detritylated using a PolyPak II cartridge (Glen Research), followed by further purification by reverse-phase HPLC (Waters XBridge C18 column, buffers: A = MeCN, B = 0.1 M TEAA (pH 7.0) + 2% MeCN, flow rate: 0.5 mL min^{-1}, temperature: 60 °C). Unmodified oligonucleotides purified by HPLC were purchased from Japan Bio Services. The concentration of the oligomers was determined based on the UV absorbance at 260 nm.[1] The synthesised DNA strands were identified by ESI-TOF mass spectrometry.

T-TFO.

HPLC retention time: 9.5 min (gradient: 7%A (0 min), 9%A (30 min)). ESI-MS: *m*/*z* cacld for $[C_{210}H_{280}N_{48}O_{139}P_{20} - 10H]^{10-}$: 630.9; found: 630.9. $\varepsilon_{260} = 1.54 \times 10^5$ M^{-1} cm^{-1}.

U^{OH}-TFO-1.

HPLC retention time: 34.8 min (gradient: 5%A (0 min), 9.5%A (45 min)). ESI-MS: *m*/*z* cacld for $[C_{209}H_{278}N_{48}O_{140}P_{20} - 9H]^{9-}$: 701.3; found: 701.3. $\varepsilon_{260} = 1.50 \times 10^5$ M^{-1} cm^{-1}.

U^{OH}-TFO-2.

HPLC retention time: 23.1 min (gradient: 5%A (0 min), 8%A (30 min)). ESI-MS: *m*/z cacld for $[C_{207}H_{274}N_{48}O_{142}P_{20} - 11H]^{11-}$: 574.0; found: 574.0. $\varepsilon_{260} = 1.42 \times 10^5$ M^{-1} cm^{-1}.

U^{OH}-TFO-3.

HPLC retention time: 22.2 min (gradient: 5%A (0 min), 8%A (30 min)). ESI-MS: *m*/*z* cacld for $[C_{207}H_{274}N_{48}O_{142}P_{20} - 10H]^{10-}$: 631.5; found: 631.5. $\varepsilon_{260} = 1.42 \times 10^5$ M^{-1} cm^{-1}.

Melting experiments

DNA triplexes were prepared by mixing **dsDNA** (1.5 μM) and a TFO (1.5 μM) in 10 mM HEPES-NaOH buffer (pH 7.0) containing 140 mM NaCl and 10 mM $MgCl_2$. After adding $GdCl_3 \cdot 6H_2O$ (Soekawa), the samples were annealed from 85 °C to 15 °C at the rate of –1.0 °C/min.

Absorbance at 260 nm was recorded on a UV-1700 spectrophotometer (Shimadzu) equipped with a TMSPC-8 temperature controller while the temperature was raised from 15 °C to 85 °C at

the rate of 0.2 °C/min. A drop of mineral oil was laid on the sample to prevent evaporation. Normalised absorbance shown in the Figures were calculated as follows:

Normalised A_{260} = {A_{260}(t °C) – A_{260}(15 °C)}/ {A_{260}(85 °C) – A_{260}(15 °C)} × 100.

The melting temperatures (T_m) were determined as an inflection point of a melting curve using a T_m analysis software LabSolutions (Shimadzu) with a 17-point adaptive smoothing program.

Native polyacrylamide gel electrophoresis (PAGE)

General. DNA triplexes were prepared with **dsDNA** labelled with 6-carboxyfluorescein (FAM) at the 5′-terminal of the purine-rich strand. The polyacrylamide gels (18%) were prepared using 50 mM HEPES-NaOH buffer (pH 7.0) containing 20 mM $MgCl_2$. The samples were mixed with 6× loading buffer (30% ethylene glycol, bromophenol blue) on ice and applied on the gel. After running at 80 V for 2 h in a cool incubator (4 or 15 °C), the gels were observed using Gel Doc EZ Imager (Bio-Rad). The bands were detected by FAM fluorescence and analysed by using Image Lab software (Bio-Rad). The yield of each product was calculated by comparing the band intensities of the triplex with that of the duplex. Averages of at least three runs are shown.

Metal titration. DNA triplexes were prepared by mixing **dsDNA** (1.5 μM) and a TFO (2.0 μM) in 10 mM HEPES-NaOH buffer (pH 7.0) containing 140 mM NaCl and 10 mM $MgCl_2$, and then varying amounts of $GdCl_3 \cdot 6H_2O$ were added. After annealing (85 °C to 15 °C, –1.0 °C/min), the samples were subjected to the native PAGE analysis (Figs. 3, S3 and S6).

Time-course analysis. DNA triplexes were prepared by mixing **dsDNA** (1.5 μM) and **U^{OH}-TFO-3** (2.0 μM) in 10 mM HEPES-NaOH buffer (pH 7.0) containing 140 mM NaCl and 10 mM $MgCl_2$. After annealing in the absence (for Fig. S7a) or in the presence of 6 equiv. of $GdCl_3$ (for Fig. S7b) (85 °C to 15 °C, –1.0 °C/min), $GdCl_3$ (6 equiv.) or EDTA (6 equiv.) was added, respectively, and the mixtures were incubated at 20 °C. The (de)hybridisation of the triplexes was analysed by native PAGE run in a 15 °C chamber to maintain the gel temperature of ca. 20 °C.

Gd^{III}-responsive reversible binding of U^{OH}-TFO-3 to the target duplex. The target duplex (**dsDNA**, 1.5 μM) was annealed (85 °C to 15 °C, –1.0 °C/min) in 10 mM HEPES-NaOH buffer (pH 7.0) containing 140 mM NaCl and 10 mM $MgCl_2$. At time = 0, **U^{OH}-TFO-3** (2 μM) was added and the mixture was incubated at 20 °C. $GdCl_3$ (6 equiv.) and EDTA (6 equiv.) were alternately added in every one hour. The binding and release of the TFO were analysed by native PAGE which was run in a 15 °C chamber to maintain the gel temperature of ca. 20 °C (Fig. 4).

UV absorption spectroscopy

DNA triplexes in the absence and presence of Gd^{III} were prepared as described above. UV spectra were recorded at 20 °C on a Hitachi U-3500 spectrophotometer with a path length of 1.0 cm (Fig. S5).

2. Supplementary figures

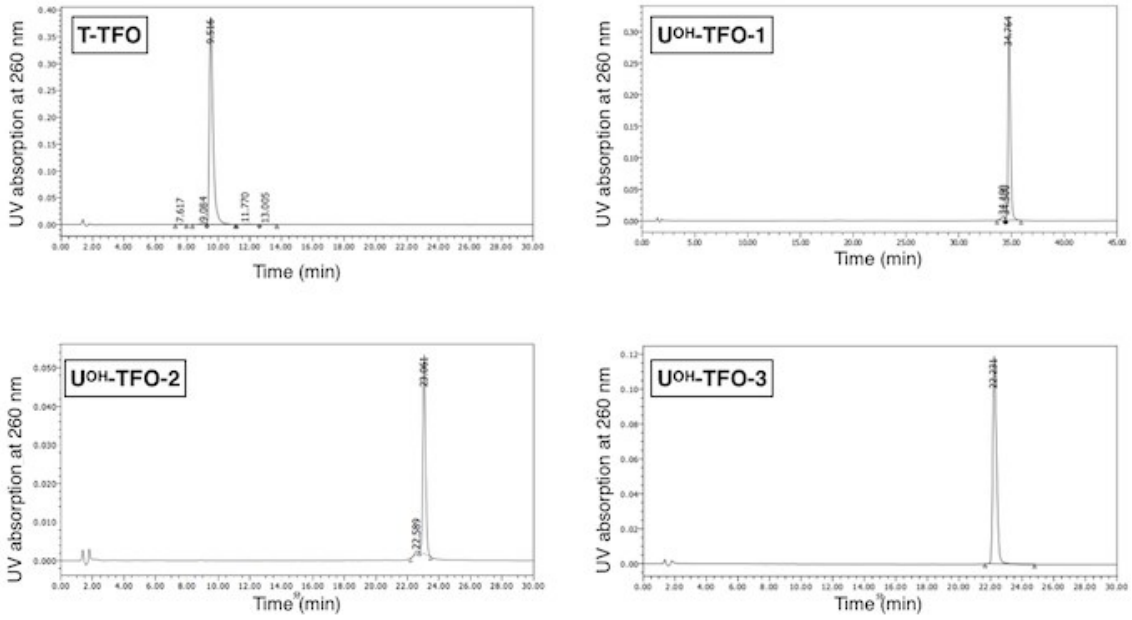

Fig. S1 HPLC profiles of the synthesised oligonucleotides. Waters XBridge C18 column, buffers: A = MeCN, B = 0.1 M TEAA (pH 7.0) + 2% MeCN, flow rate: 0.5 mL/min, temperature: 60 °C.

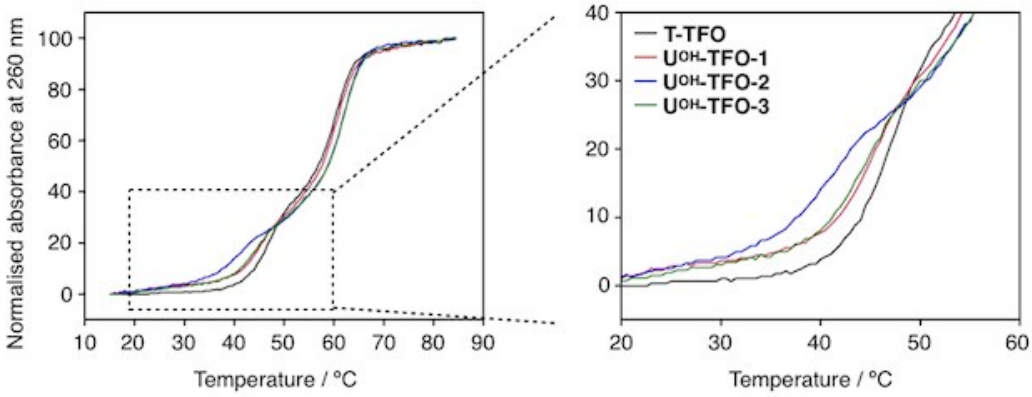

Fig. S2 Melting curves of DNA triplexes consisting of **U^{OH}**-containing TFOs and the target duplex (**dsDNA**). [**dsDNA**] = [TFO] = 1.5 μM in 10 mM HEPES-NaOH buffer (pH 7.0), 140 mM NaCl, 10 mM $MgCl_2$, 0.2 °C/min.

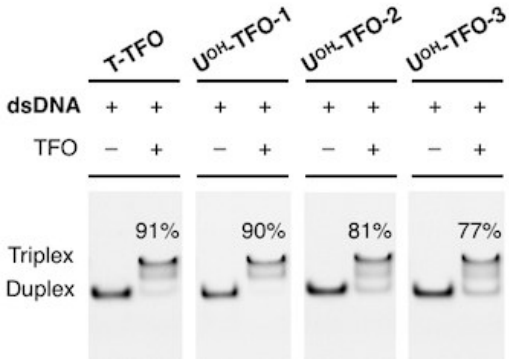

Fig. S3 Native PAGE analyses of the mixtures of **U^{OH}**-containing TFOs and the target duplex (**dsDNA**). [**dsDNA**] = 1.5 μM, [TFO] = 0, 2.0 μM in 10 mM HEPES-NaOH buffer (pH 7.0), 140 mM NaCl, 10 mM $MgCl_2$. 18% Gel, 50 mM HEPES buffer (pH 7.0), 20 mM $MgCl_2$, at 4 °C. FAM detection.

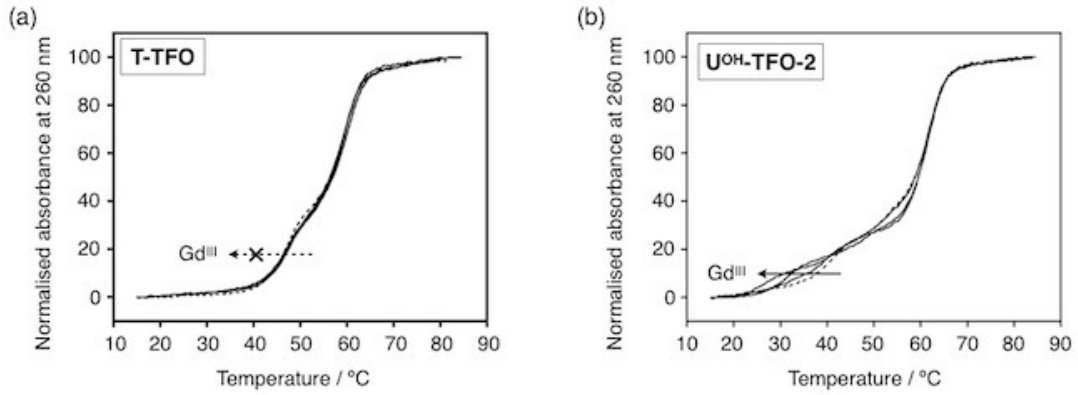

Fig. S4 Melting curves of DNA triplexes consisting of (a) **T-TFO** and (b) **U^{OH}-TFO-2** in the absence or presence of Gd^{III} ions. [**dsDNA**] = [TFO] = 1.5 μM, [$GdCl_3$]/[TFO] = 0, 1, 3 or 6 in 10 mM HEPES-NaOH buffer (pH 7.0), 140 mM NaCl, 10 mM $MgCl_2$, 0.2 °C/min.

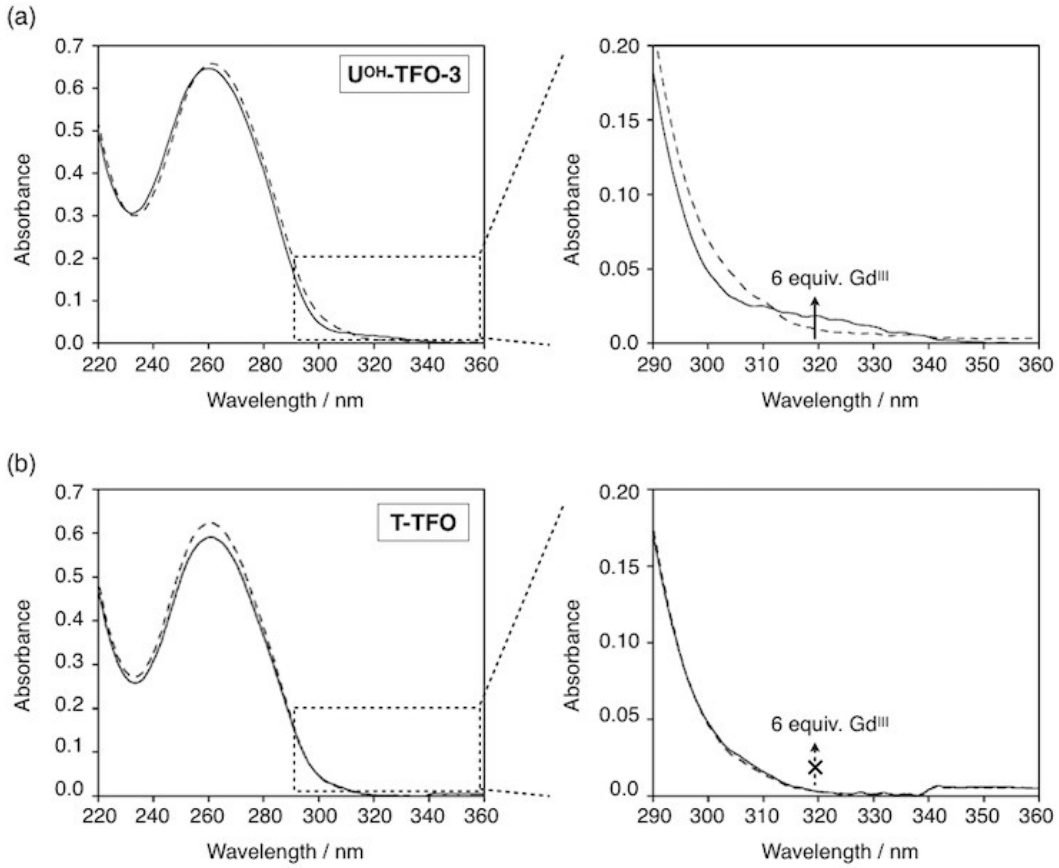

Fig. S5 UV absorption spectra of a DNA triplex consisting of (a) **U^{OH}-TFO-3** or (b) **T-TFO** in the absence or presence of Gd^{III} ions. [**dsDNA**] = [TFO] = 1.5 μM, $[GdCl_3]$/[TFO] = 0, 6 in 10 mM HEPES-NaOH buffer (pH 7.0), 140 mM NaCl, 10 mM $MgCl_2$. *l* = 1.0 cm, at 20 °C.

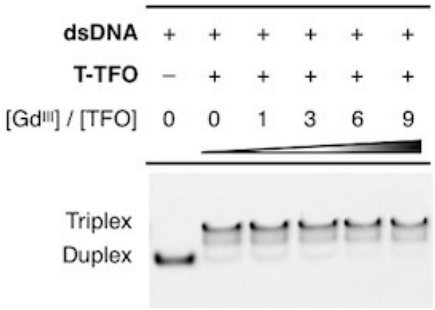

Fig. S6 Native PAGE analysis of the DNA triplex consisting of **T-TFO** in the presence of Gd^{III} ions [dsDNA] = 1.5 μM, [**T-TFO**] = 0, 2.0 μM, $[GdCl_3]$/[**T-TFO**] = 0, 1, 3, 6 or 9 in 10 mM HEPES-NaOH buffer (pH 7.0), 140 mM NaCl, 10 mM $MgCl_2$. 18% Gel, 50 mM HEPES buffer

(pH 7.0), 20 mM $MgCl_2$, at 4 °C. FAM detection.

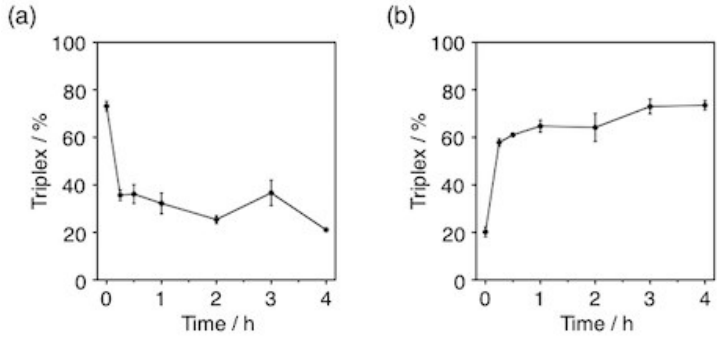

Fig. S7 Time-course analyses of Gd^{III}-dependent dissociation and association of DNA triplexes consisting of **U^{OH}-TFO-3**. (a) Dissociation of the triplex triggered by the addition of Gd^{III}. (b) Triplex formation triggered by the removal of Gd^{III} using EDTA. [**dsDNA**] = 1.5 μM, [**U^{OH}-TFO-3**] = 2.0 μM, [$GdCl_3$] = 12 μM, [EDTA] = 0, 12 μM in 10 mM HEPES-NaOH buffer (pH 7.0), 140 mM NaCl, 10 mM $MgCl_2$. The yields were determined by native PAGE analyses. Error bars indicate the standard errors.

3. Supplementary tables

Table S1. Melting temperatures of the DNA triplexes consisting of **U^{OH}**-containing TFOs in the absence and presence of Gd^{III} ions[a].

	Melting transition	Gd^{III}-free	6 eq. of Gd^{III}	
		T_m / °C	T_m / °C	ΔT_m / °C[b]
dsDNA + T-TFO	1st (T_{m1})	48.3 ± 0.9	47.6 ± 0.5	–0.8 ± 1.1
	2nd (T_{m2})	61.6 ± 0.6	60.7 ± 0.4	–0.8 ± 0.7
dsDNA + U^{OH}-TFO-1	1st (T_{m1})	45.6 ± 0.1	41.3 ± 0.1	–4.3 ± 0.1
	2nd (T_{m2})	60.9 ± 0.1	59.8 ± 0.2	–1.1 ± 0.2
dsDNA + U^{OH}-TFO-2	1st (T_{m1})	41.8 ± 0.6	26.2 ± 0.7	–15.6 ± 0.9
	2nd (T_{m2})	61.9 ± 0.5	61.1 ± 0.2	–0.8 ± 0.5
dsDNA + U^{OH}-TFO-3	1st (T_{m1})	45.2 ± 0.0	29.7 ± 1.1	–15.6 ± 1.1
	2nd (T_{m2})	62.0 ± 0.2	62.3 ± 0.4	+0.3 ± 0.5

[a] Average T_m values of at least 3 runs are listed. Standard errors are also shown. [b] ΔT_m represents the difference in the T_m values relative to that of the Gd^{III}-free DNA triplexes.

4. References

1. Y. Takezawa, K. Nishiyama, T. Mashima, M. Katahira and M. Shionoya, *Chem. – Eur. J.*, 2015, **21**, 14713–14716.
2. J. Fujimoto, L. Tran and L. C. Sowers, *Chem. Res. Toxicol.*, 1997, **10**, 1254–1258.
3. M. L. Morningstar, D. A. Kreutzer and J. M. Essigmann, *Chem. Res. Toxicol.*, 1997, **10**, 1345–1350.