Supporting Information for

Enhancement in RNase H activity of DNA/RNA hybrid duplex using artificial cationic oligopeptides

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General information

The synthesis of oligopeptides was conducted according to the procedures described in our previous report.[8b] DNA and RNA oligomers were purchased from Japan Bio Services Co., Ltd.

UV melting analyses

The measurement for absorbance vs. temperature profiles were obtained an eight-sample cell changer in quartz cells with a 1-cm path length. All the experiments were conducted in 10 mM phosphate buffer containing 100 mM NaCl at pH 7.0. The concentration of the duplex was 4 μM and that of oligopeptides was 0 or 4 μM. The UV absorbance was monitored at 260 nm along with the temperature. First, the samples containing oligonucleotides were rapidly heated to 95 °C, maintained at this temperature for 10 min, and finally allowed to cool to room temperature at a rate of 1.0 °C/min. Cationic oligopeptides were then added to the solution; these samples were further cooled to 10 °C at a rate of 0.5 °C/min, and the process of the dissociation of the hybrid duplex was recorded by heating the samples to 95 °C at a rate of 0.5 °C/min.

Evaluation of RNase A resistance

All experiments were conducted using 100 nM of the DNA/RNA hybrid and 200 nM of the cationic oligopeptides in 10 mM Tris buffer containing 100 mM NaCl (pH 7.3) at 30 °C. To a solution of duplex with or without cationic oligopeptides, a solution of 100 μg/mL of RNase A from bovine pancreas in Tris buffer was added to produce a final concentration of 0.50 μg/mL. The fluorescence intensity was recorded (at 10 s intervals) for 3600 s, 10 s after the addition of RNase A. The following instrument settings were used: Ex/Em, 490 nm/520 nm; response, 2 sec; band width (Ex), 1 nm; band width (Em), 3 nm.

Evaluation of RNase H activity

All experiments were conducted using 100 nM of the DNA/RNA hybrid and 200 nM of the cationic oligopeptides in 10 mM Tris buffer containing 100 mM NaCl (pH 7.2) at 20 °C. To a solution of duplex with or without cationic oligopeptides, 10U/μL of RNase H from *Escherichia coli* in Tris buffer (2 μL) was added to give a final concentration of 6.7 U/mL. The fluorescence intensity was recorded (at 10 s intervals) for 3600 s, 10 s after addition of RNase A. The following instrument settings were used: Ex/Em, 490 nm/520 nm; response, 2 sec; band width (Ex), 1 nm; band width (Em), 3 nm.

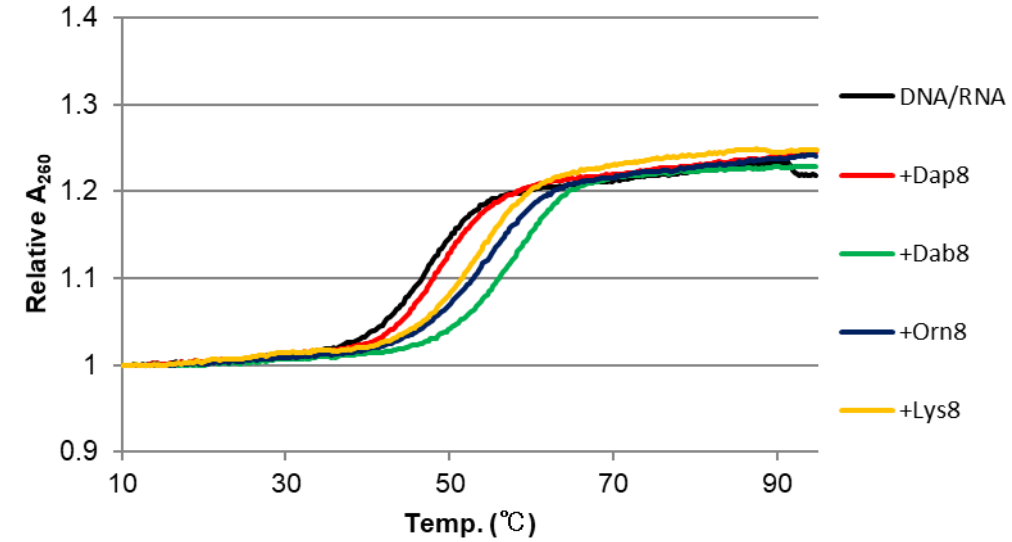

Figure S1. UV melting curves of DNA/RNA hybrid duplex (d(CAGT)$_3$/ r(ACUG)$_3$) in the absence and presence of oligoaminopeptides.

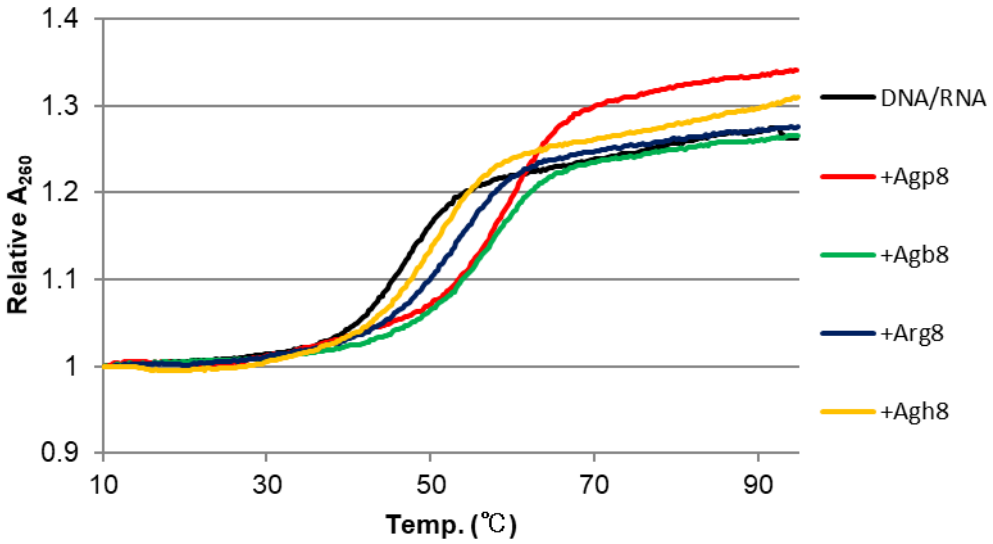

Figure S2. UV melting curves of DNA/RNA hybrid duplex (d(CAGT)$_3$/ r(ACUG)$_3$) in the absence and presence of oligoguanidinopeptides.

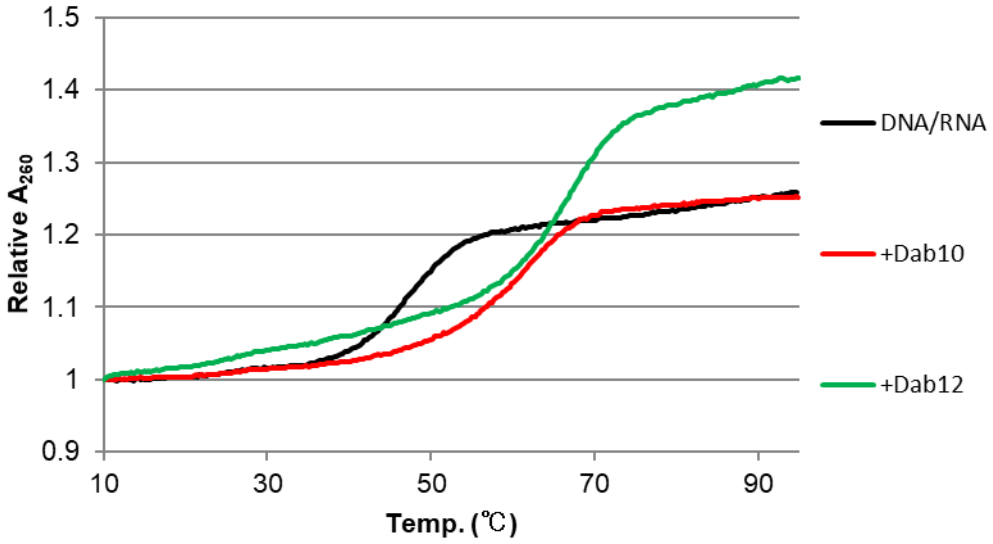

Figure S3. UV melting curves of DNA/RNA hybrid duplex (d(CAGT)$_3$/ r(ACUG)$_3$) in the absence and presence of Dab10 and Dab12.

CD spectra were recorded with a Jasco J-725 using a 1 mm cell at wavelength of 320 to 200 nm. All the experiments were conducted in 10 mM phosphate buffer containing 100 mM NaCl at pH 7.0 and the concentration of the dsRNA was 5 μM in the absence and presence of oligopeptides at 20 °C. The following instrument settings were used: Resolution, 0.1-0.2 nm; Band width, 2.0 nm; response, 2-4 sec; speed, 10 nm/min; accumulation, 4.

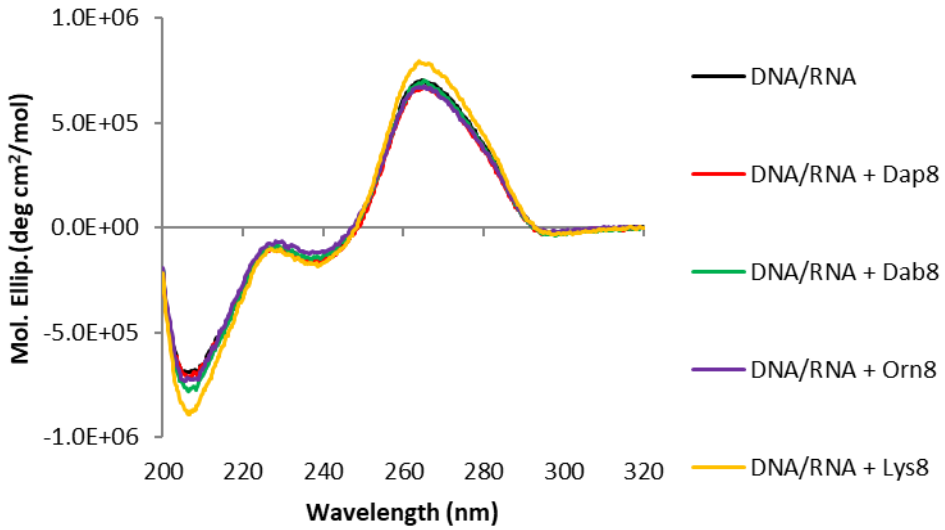

Figure S4. CD spectra of DNA/RNA hybrid duplex (d(CAGT)$_3$/ r(ACUG)$_3$) in the absence and presence of oligoaminopeptides.

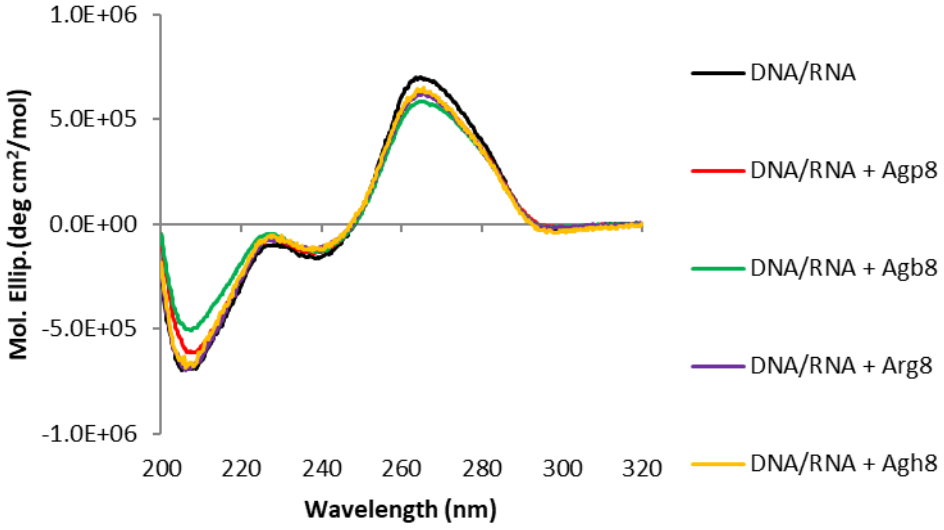

Figure S5. CD spectra of DNA/RNA hybrid duplex (d(CAGT)$_3$/ r(ACUG)$_3$) in the absence and presence of oligoguanidinopeptides.

Table S1 K_d values of the cationic peptides for the binding to FAM-labeled 12mer DNA/RNA hybrid duplex (d(CAGT)$_3$/ FAM-r(ACUG)$_3$,) at 20 °C in 10 mM phosphate buffer containing 100 mM NaCl and 0.2% Tween 20 (pH 7.0). These experiments were conducted according to the method described in our previous report.[16]

Entry	Peptide	$K_d/10^{-8}$ M
1	Dab8	1.7 ± 0.7
2	Agp8	3.1 ± 0.9

[16] R. I. Hara, Y. Maeda, T. Sakamoto, T. Wada, *Org. Biomol. Chem.*, 2017, **15**, 1710.

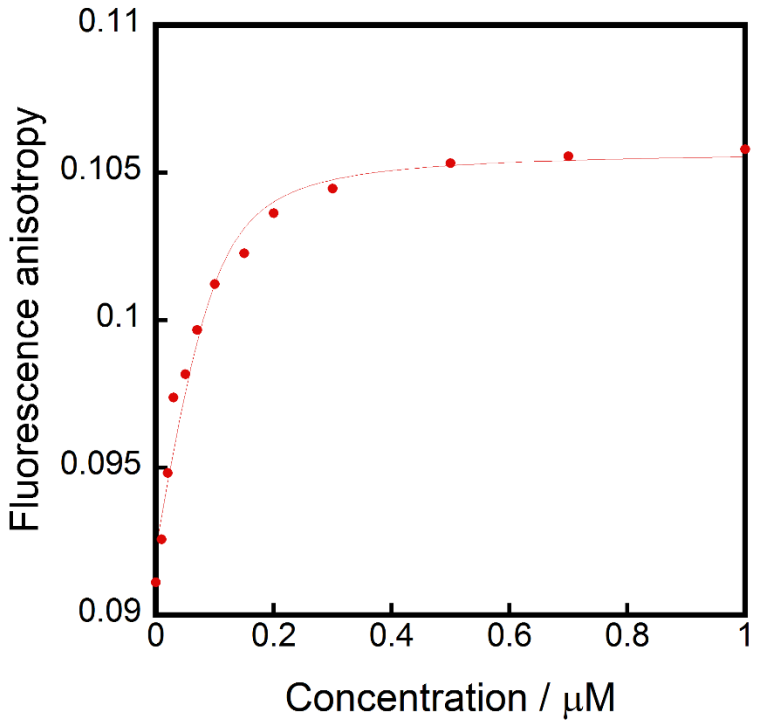

Figure S6. Fluorescence anisotropy of 100 nM d(CAGT)$_3$/ FAM-r(ACUG)$_3$ was titrated by increasing concentration of Dab8.

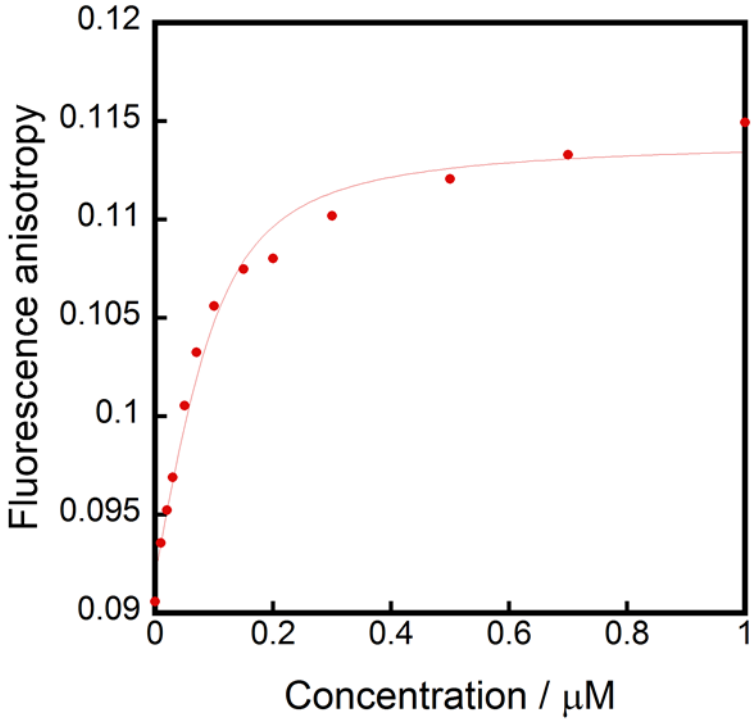

Fig. S7. Fluorescence anisotropy of 100 nM d(CAGT)$_3$/ FAM-r(ACUG)$_3$ was titrated by increasing concentration of Agp8.

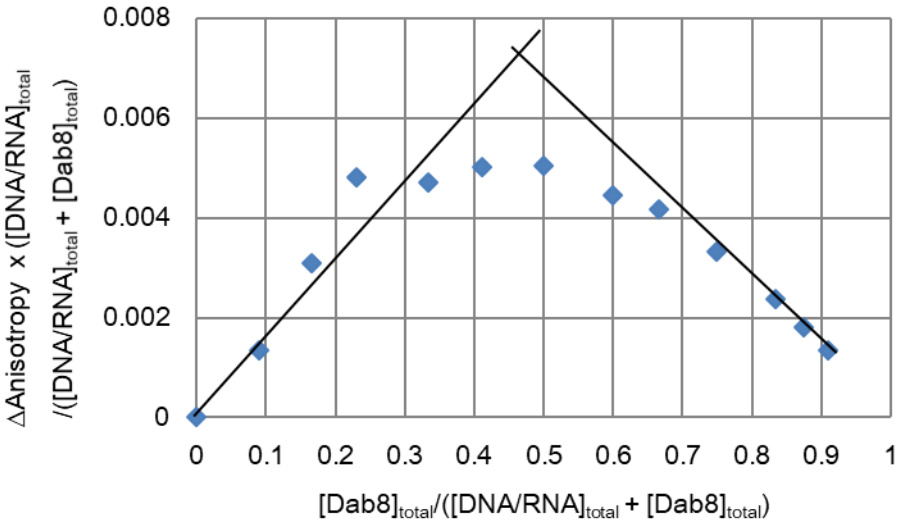

Figure S8. Job plots for estimating binding ratio of Dab8-DNA/RNA. $N = 0.9$.

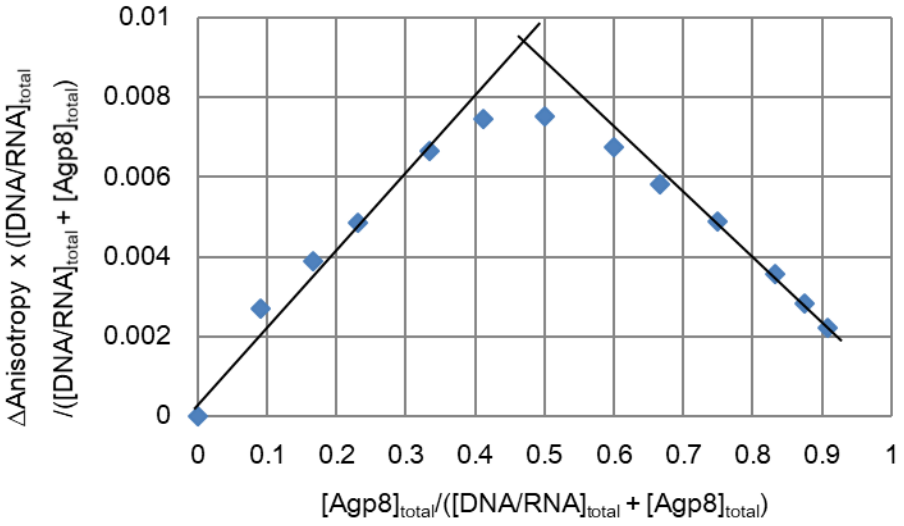

Figure S9. Job plots for estimating binding ratio of Agp8-DNA/RNA. $N = 0.9$.

Table S2. Apparent $t_{1/2}$ in the RNase A cleavage reaction of DNA/RNA hybrids in the absence or presence of cationic oligopeptides (Figure 2).

Entry	Peptide	Apparent $t_{1/2}$ / min
0	none	3.5
1	Dap8	1.7
2	Dab8	11.0
3	Orn8	6.3
4	Lys8	7.0
5	Agp8	>18.3
6	Agb8	13.3
7	Arg8	12.3
8	Agh8	8.0

Table S3. Apparent $t_{1/2}$ in the RNase A cleavage reaction of DNA/RNA hybrids in the absence or presence of cationic oligopeptides (Figure 3).

Entry	Peptide	Apparent $t_{1/2}$ / min
0	none	9.8
1	Dap8	9.7
2	Dab8	5.0
3	Orn8	10.3
4	Lys8	10.2
5	Agp8	13.7
6	Agb8	10.5
7	Arg8	12.3
8	Agh8	12.2
9	Dab10	5.3
10	Dab12	7.8