Bioorganic & Medicinal Chemistry 21 (2013) 1717–1723

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and properties of cationic oligopeptides with different side chain lengths that bind to RNA duplexes

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ARTICLE INFO

Article history:
Received 4 January 2013
Revised 22 January 2013
Accepted 23 January 2013
Available online 1 February 2013

Keywords:
RNA-binding peptide
Cationic peptide
Artificial peptide
RNAi drug
DDS

ABSTRACT

A series of artificial peptides bearing cationic functional groups with different side chain lengths were designed, and their ability to increase the thermal stability of nucleic acid duplexes was investigated. The peptides with amino groups selectively increased the stability of RNA/RNA duplexes, and a relationship between the side chain length and the melting temperature (T_m) of the peptide–RNA complexes was observed. On the other hand, while peptides with guanidino groups exhibited a similar tendency with respect to the peptide structure and thermal stability of RNA/RNA duplexes, those with longer side chain lengths, such as L-2-amino-4-guanidinobutyric acid (Agb) or L-arginine (Arg) oligomers, stabilized both RNA/RNA and DNA/DNA duplexes, and those with shorter side chain lengths exhibited a higher ability to selectively stabilize RNA/RNA duplexes. In addition, peptides were designed with different levels of flexibility by introducing glycine (Gly) residues into the L-2-amino-3-guanidinopropionic acid (Agp) oligomers. It was found that insertion of Gly did not affect the thermal stability of the peptide–RNA complexes, but an alternate arrangement of Gly and Agp apparently decreased the thermal stability. Therefore, in the Agp oligomer, consecutive Agp sequences are essential for increasing the stability of RNA/RNA duplexes.

1. Introduction

In recent years, increased attention has been paid to the development of nucleic acid drugs. For example, antisense oligonucleotides and RNA interference drugs (RNAi drugs) are well known molecular tools for the regulation of gene expression, in which their mechanisms of action are based on sequence specific interactions.[1] The RNAi drugs act on the target mRNA in a sequence selective manner. Thus, RNAi drugs are attractive because of their high selectivity for the target and their shorter drug development time. Therefore, siRNAs have been widely studied for therapeutic applications; however, such RNA molecules are not sufficiently effective because of their low-membrane permeability and instability in cells. To stabilize oligonucleotides against metabolic degradation, a number of chemical modifications have been proposed,[2] and RNAi drugs generally consist of double stranded RNAs (dsRNAs) with chemical modifications. A proper modification of an RNA molecule increases its stability in cells and improves its pharmacokinetic properties.[3] Another strategy for stabilizing siRNA is the use of molecules that can non-covalently bind to RNA to protect it from attack by nucleases. For example, a fusion protein of the peptide transduction domain-dsRNA binding domain was shown to effectively transport RNAi drugs into primary cells.[4] In this case, the increased thermal stability of the dsRNAs also increased their stability in cells.[5] We are thus attempting to develop RNA/RNA duplex-binding molecules that are useful as drug delivery systems (DDSs) for siRNAs.

A wide variety of RNA-binding molecules have been reported, such as aminoglycosides[6] and RNA-binding proteins.[7] In paticular, chemically modified peptides bearing different methylene lengths in the arginine or lysine residues possess diverse affinities to RNAs.[8] In our previous study, α-(1 → 4)-linked-2,6-diamino-2,6-dideoxy-D-glucopyranose oligomers were synthesized, and their highly RNA-selective binding ability was demonstrated.[9] These results suggested that the geometry of the cationic groups, particularly the distance between the cationic groups, affects the affinity and selectivity of the oligomers for nucleic acid duplexes. Therefore, in this study, we designed a series of cationic oligopeptides to reveal the relationship between the geometry of the cationic groups and the affinity of the peptides for nucleic acids. In contrast to oligosaccharide derivatives, peptides can easily be synthesized, which is advantageous for obtaining a systematic series of molecules. They can also be readily connected with other functional groups such as transporter molecules.[10] Therefore, RNA duplex-binding peptides are useful tools for the development of drug delivery systems (DDSs) for RNAi drugs. The L-arginine (Arg) oligomers designed from the HIV Tat peptide are well known for their high-membrane permeability[11] and the Arg 15mer has been used as an RNAi transporter by forming a complex with RNA.[12]

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http://dx.doi.org/10.1016/j.bmc.2013.01.053

However, the activity varies with the side chain length,[13] and thus a more detailed study of the relationship between the structure and activity is important for the development of effective carriers for RNAi drugs. Thus, to investigate the structural effects on binding ability, we designed and synthesized a number of cationic oligopeptides bearing different side chain lengths to control the distance between the cationic functional groups and peptides with different flexibility by incorporating glycine (Gly).

2. Results and discussion

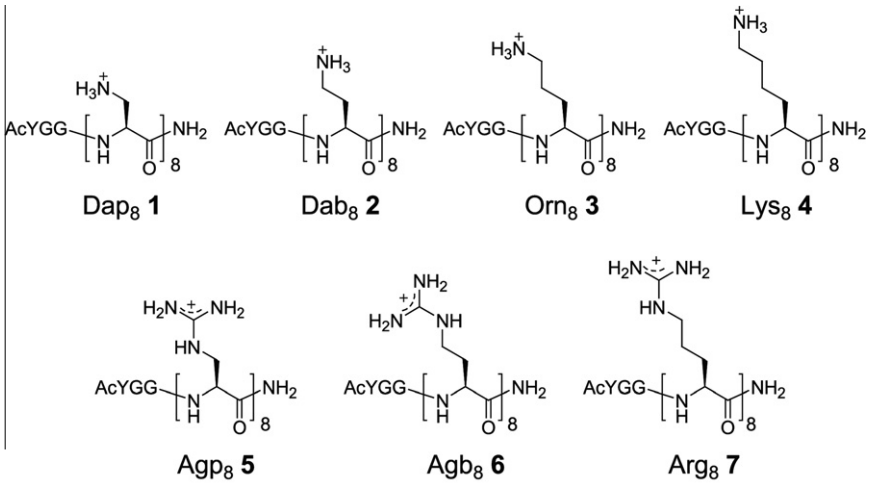
2.1. Design of the peptides

A series of cationic oligopeptides was designed (Fig. 1). All peptides contained a unit of *N*-acetyl-L-tyrosine with two glycine residues at the N-terminus for UV detection and quantification. In this study, the 12mer of nucleic acid duplexes was used as a model to estimate the interaction with peptides bearing four or eight cationic groups because four pairs of phosphate groups are aligned on the inward portion of the major groove of the 12mer of A-type nucleic acid duplexes. First, to compare the effects of the distance between cationic groups, peptides with different side chain lengths were designed. Peptides were synthesized with amino groups (L-2,3-diaminopropionic acid (Dap), L-2,4-diaminobutyric acid (Dab), L-ornithine (Orn), and L-lysine (Lys)) and guanidino groups (L-2-amino-3-guanidinopropionic acid (Agp), L-2-amino-4-guanidinobutyric acid (Agb), and L-arginine (Arg)). In some molecules, several glycine units were also inserted into an Agp octamer to increase the flexibility of the peptides. Peptides with alternate arrangements were also designed to change the position and combination of the functional groups. These peptides were based on the Agp oligomer and other amino acids such as glycine (Gly), L-serine (Ser), and L-asparagine (Asn). Glycine was chosen because of its high flexibility. Ser and Asn were chosen because they are well known in RNA-binding proteins to form hydrogen bonds with the phosphate groups of RNA.[14]

On the basis of molecular mechanics calculations with a GB/SA water solvation model,[15] a cationic oligopeptide 8mer consisting of Dab can bind to the major groove of an A-type RNA/RNA duplex 12mer, in which all the protonated amino groups of the peptide form the hydrogen bonds to the phosphate anions of the duplex (Fig. 2). Therefore, the electrostatic interaction and hydrogen bonding would be important for the binding of cationic oligopeptides to RNA/RNA duplexes.

2.2. Melting temperature (T_m) analysis

The T_m values of the RNA duplexes were measured both in the absence and presence of an equal amount of peptides. All measurements were performed under-physiological conditions with 10 mM phosphate buffer containing 100 mM NaCl at pH 7.0. Figure 2 shows the melting temperature enhancements and Table 1 lists the T_m values for the self complementary RNA 12mer r(CGCGAAUUCGCG)$_2$ in the absence and presence of an equal amount of peptides with amino groups. In this study, the peptides were added into the solution of nucleic acid duplexes after annealing to avoid the aggregation of the peptides at high temperature. The T_m values were affected by the side chain length, with Dab_8 (**2**) showing the highest T_m value. Comparing the Dab_8 (**2**), Orn_8 (**3**), and Lys_8 (**4**), the T_m value increased as the side chain length



Peptides with amino groups			Peptides with flexible main chains			
Dap_8	:	Ac-YGG-Dap_8-NH_2 **1**	Agp_8G1	:	Ac-YGG-Agp_4-G-Agp_4-NH_2	**8**
Dab_8	:	Ac-YGG-Dab_8-NH_2 **2**	Agp_8G2	:	Ac-YGG-Agp_3-G-Agp_2-G-Agp_3-NH_2	**9**
Orn_8	:	Ac-YGG-Orn_8-NH_2 **3**	Agp_8G3	:	Ac-YGG-Agp_2-G-Agp_2-G-Agp_2-G-Agp_2-NH_2	**10**
Lys_8	:	Ac-YGG-Lys_8-NH_2 **4**	Agp_8A2	:	Ac-YGG-Agp_3-A-Agp_2-A-Agp_3-NH_2	**11**
Peptides with guanidino groups			Agp_8P2	:	Ac-YGG-Agp_3-P-Agp_2-P-Agp_3-NH_2	**12**
Agp_8	:	Ac-YGG-Agp_8-NH_2 **5**	**Peptides with alternate arrangement**			
Agb_8	:	Ac-YGG-Agb_8-NH_2 **6**	AgpG	:	Ac-YGG-(Agp-G)$_4$-NH_2	**13**
Arg_8	:	Ac-YGG-Arg_8-NH_2 **7**	AgpS	:	Ac-YGG-(Agp-S)$_4$-NH_2	**14**
			AgpN	:	Ac-YGG-(Agp-N)$_4$-NH_2	**15**

Figure 1. Structures and sequences of cationic peptides.

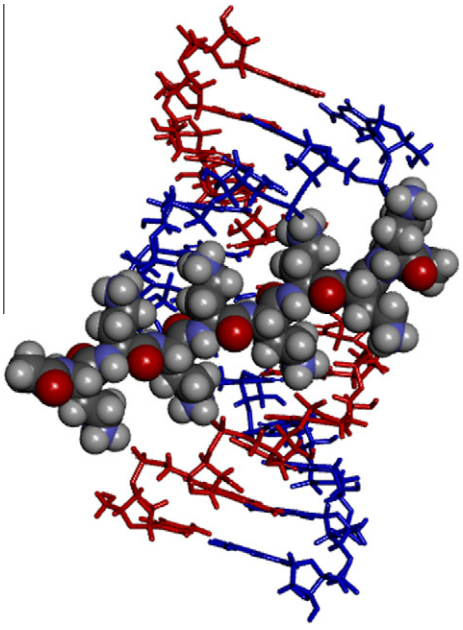


Figure 2. Molecular model of L-2,4-diaminobutylic acid (Dab) 8mer binding to A-type RNA–RNA duplex (12mer).

Table 1
Thermal melting points (T_m in °C) for oligonucleotide duplex (in the absence and presence of peptides with amino groups[a])[b]

Peptide	RNA/RNA	ΔT_m	DNA/DNA	ΔT_m
None	60.7		48.2	
Dap_8	60.2	−0.5	50.8	2.6
Dab_8	74.7	14.0	49.5	1.3
Orn_8	74.2	13.5	51.3	3.1
Lys_8	68.1	7.4	49.7	1.5

[a] Peptide concentration 4 μM.
[b] Buffer (10 mM phosphate buffere for pH 7.0), NaCl (100 mM), together with each oligonucleotide strand (4 μM). T_m values are reported at the means of duplicate measurements.

decreased. These results suggest that the distance between the amino groups in Dab_8 (**2**) fits to the distance of the opposing phosphate groups in the RNA duplexes. This tendency has been previously reported for cationic oligopeptides with D-amino acids.[6] On the other hand, Dap_8 (**1**) did not stabilize the RNA/RNA duplexes. This differing behavior may result because the distance of the amino groups in Dap_8 is too short to interact with the phosphate groups in the RNA/RNA duplexes. In fact, none of the peptides with amino groups increased the thermal stability of the DNA/DNA duplex d(CGCGAATTCGCG)$_2$ (Fig. 3). The distance between the phosphate groups in the major groove of a DNA/DNA duplex is twice that in an RNA/RNA duplex. Therefore, none of the peptides were able to interact with the DNA/DNA duplexes. In the minor groove of a DNA/DNA duplex, the interstrand phosphate groups are much closer than those in the major groove. However, the phosphate groups facing outward of the minor groove are unfavorable to form hydrogen bonds with the protonated amino groups of cationic peptides.

The peptides with guanidino groups (**5**, **6**, **7**) exhibited the same tendency as the peptides with the amino groups (Fig. 4, Table 2) for the RNA/RNA duplexes. Peptides with shorter side chain lengths had higher T_m values. This result indicates that the position of guanidino groups in Agp_8 (**5**) also fits well with the RNA duplex structure. While the distance between the functional groups is similar in the peptides with amino and guanidino groups, the peptides with

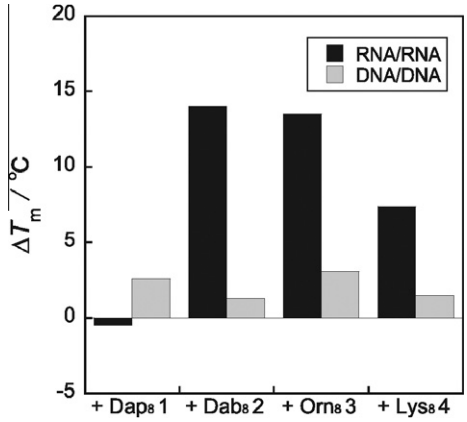


Figure 3. Melting temperature enhancements for the RNA/RNA and DNA/DNA duplexes at pH 7.0 in the presence of peptides **1**–**4**. Differences in the thermal melting points (ΔT_m) are given for the nucleic acid duplexes in the presence of equimolar amounts of peptide relative to the duplex alone. The dark gray columns represent ΔT_m for RNA/RNA duplex, and light gray columns represent ΔT_m values for DNA/DNA duplex.

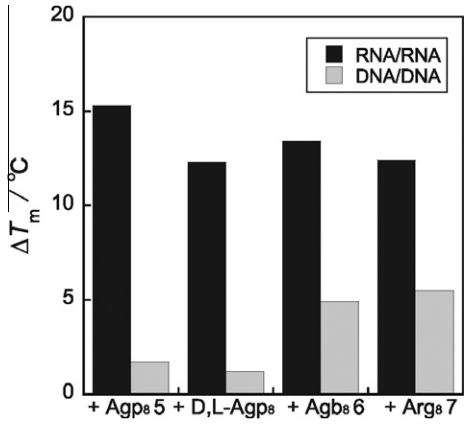


Figure 4. Melting temperature enhancements for the RNA/RNA and DNA/DNA duplexes at pH 7.0 in the presence of peptides **5**–**7**. Differences in the thermal melting points (ΔT_m) are given for the nucleic acid duplexes in the presence of an equal amount of peptide relative to the duplex alone. The dark gray columns represent ΔT_m for RNA/RNA duplex, and light gray columns represent ΔT_m values for DNA/DNA duplex.

Table 2
Thermal melting points (T_m in °C) for oligonucleotide duplex (in the absence and presence of peptides with guanidino groups[a])[b]

Peptide	RNA/RNA	ΔT_m	DNA/DNA	ΔT_m
None	60.7		48.2	
Agp_8	76.9	16.2	49.9	1.7
D-Agp_8	75.8	15.1	48.9	0.7
D,L-Agp_8	73.0	12.3	49.4	1.2
Agb_8	74.1	13.4	53.1	4.9
Arg_8	73.1	12.4	53.7	5.5

[a] Peptide concentration 4 μM.
[b] Buffer (10 mM phosphate buffere for pH 7.0), NaCl (100 mM), together with each oligonucleotide strand (4 μM). T_m values are reported at the means of duplicate measurements.

the guanidino groups exhibited higher T_m values. These different T_m values can be attributed to the different features of the functional groups. The guanidino group is known to have a stronger interaction with phosphate groups than amino groups, and thus the peptides with guanidino groups showed a stronger interaction

with the nucleic acid duplexes. However, in the case of the DNA/DNA duplex, the tendency changed because of the differences in the guanidino and amino functional groups. For the peptides with guanidine groups, those with longer side chain lengths had higher T_m values. This result may also be due to the fact that the guanidino groups strongly interact with the phosphate groups.

The different side chain lengths of the peptides resulted in different thermal stabilities of the nucleic acid duplexes. The duplex stability may also be affected by the flexibility of the peptides. Thus, to investigate the effect of incorporation of flexible residues into the peptide backbone, glycine was inserted into Agp_8 (**5**) to increase the flexibility of the main chain (**8**, **9**, **10**). Interestingly, it was found that an increase in the flexibility of the main chain did not affect the thermal stability of the peptide–RNA complexes (Fig. 5, Table 3). These results suggest that the stabilization of the RNA duplex with Agp_8 (**5**) is mainly attributed to enthalpic factors. In addition, insertion of L-alanine (Ala) or L-proline (Pro) into Agp_8 (**5**) also did not affect the thermal stability of the RNA duplexes (**11**, **12**). If the peptides invade the major groove, the redundant amino acid residues will give rise to steric hindrance and decrease the thermal stability. Therefore, these results suggest that the peptides interact with the surface of the nucleic acids.

Peptides with alternate arrangements also did not stabilize the RNA duplexes (Fig. 6, Table 4). Peptides consisting of Agp and Gly in an alternate arrangement, AgpG (**13**) did not have any effects on the RNA/RNA duplex. On the other hand, Agp_4 showed an appreciable stabilization effect for the RNA/RNA duplex. Therefore, a consecutive Agp sequence is effective for RNA/RNA duplex stabilization. Furthermore, Agp_8G3 (**10**), in which four Agp_2 units are connected, has the same affinity as Agp_8; thus, the Agp_2 structural unit is effective for the interaction with RNA/RNA duplexes. In Agp oligomers with a consecutive sequence, the guanidino groups are located on both sides of the peptide backbone, and this alignment is identical to that required for interaction between the guanidino groups and the phosphates in the major groove of RNA duplexes. However, peptides with a combination of guanidino and hydroxy or amide groups (AgpS (**14**) and AgpN (**15**), respectively) had no effects on the thermal stability of RNA duplexes. These results also suggest that a consecutive arrangement of cationic amino acids is essential for effective interaction with the phosphates of RNA duplexes.

The effect of chirality was also examined. The Agp oligomers consisting of all-L- and all-D-amino acids had similar T_m values,

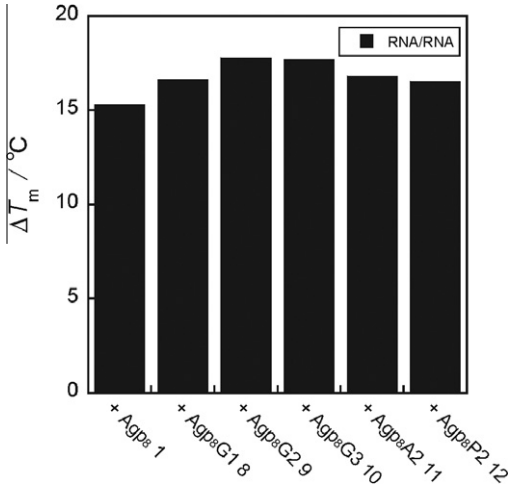


Figure 5. Melting temperature enhancements for the RNA/RNA duplexes at pH 7.0 in the presence of peptides **1**, **8**–**12**. Differences in the thermal melting points (ΔT_m) are given for the nucleic acid duplexes in the presence of an equal amount of peptide relative to the duplex alone.

Table 3
Thermal melting points (T_m in °C) for oligonucleotide duplex (in the absence and presence of peptides with flexible main chian[a])[b]

Peptide	RNA/RNA	ΔT_m
None	60.9	
Agp_8	77.5	16.6
Agp_8G1	77.5	16.6
Agp_8G2	78.8	17.9
Agp_8G3	78.6	17.7
Agp_8A2	77.7	16.8
Agp_8P2	77.4	16.5

[a] Peptide concentration 4 μM.
[b] Buffer (10 mM phosphate buffere for pH 7.0), NaCl (100 mM), together with each oligonucleotide strand (4 μM). T_m values are reported at the means of duplicate measurements.

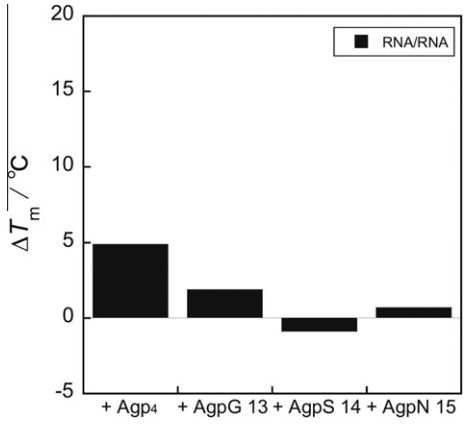


Figure 6. Melting temperature enhancements for the RNA/RNA duplexes at pH 7.0 in the presence of peptides **13**–**15**. Differences in the thermal melting points (ΔT_m) are given for the nucleic acid duplexes in the presence of equimolar amounts of peptide relative to the duplex alone.

Table 4
Thermal melting points (T_m in °C) for oligonucleotide duplex (in the absence and presence of peptides with alternate arrangements[a])[b]

Peptide	RNA/RNA	ΔT_m
None	60.3	
Agp_4	65.2	4.9
AgpG	62.2	1.9
AgpS	58.9	−1.4
AgpN	60.5	0.2

[a] Peptide concentration 4 μM.
[b] Buffer (10 mM phosphate buffere for pH 7.0), NaCl (100 mM), together with each oligonucleotide strand (4 μM). T_m values are reported at the means of duplicate measurements.

but the peptides having D/L-alternate arrangements slightly had lower thermal stability. A decrease in the thermal stability induced by a heterochiral backbone was also reported for chiral PNA.[16]

2.3. CD spectroscopy

The structures of the peptides and the RNA–peptide complexes in solution were analyzed using circular dichroism (CD) spectroscopy. On the basis of molecular mechanics calculations,[15] the amino groups and guanidino groups of the peptides, particularly in Dap_8 (**2**) and Agp_8 (**5**), can form intramolecular hydrogen bonds with the amido groups in the main chain. However, in the absence of nucleic acid duplexes, the spectra of all the peptides indicated the presence of random coils.[17] Therefore, the effect of the secondary structures of the peptides was negligible in these cases. The

structures of the RNA–peptide complexes were also analyzed. Because the peptides showed a variety of T_m values depending on the side chain length and the nature of the cationic functional groups, there existed not only electrostatic interactions and hydrogen bonding, but also structural factors. However, for both the RNA and DNA duplexes, no appreciable structural changes in the nucleic acids were observed following the addition of the peptides (Figs. 7 and 8). The CD spectra of the RNA and DNA duplexes in the presence and absence of peptides were typical for A-type and B-type helices, respectively.

2.4. ITC measurement

Thermodynamic analysis of the peptide–nucleic acid interactions was carried out using isothermal titration calorimetry (ITC) measurements. The duplex concentration was 2.5 times higher than that used for the UV melting analyses because the amount of heat generated during the binding between the nucleic acid duplexes and the oligocationic peptides was expected to be insufficient for calculation of the thermodynamic parameters at the lower concentration. Figures 9, S8,[17] and S9[17] show the preliminary results for the ITC titration of the peptides with the self complementary RNA/RNA duplex r(CGCGAAUUCGCG)$_2$ and DNA/DNA duplex d(CGCGAATTCGCG)$_2$. In addition to electrostatic interactions and hydrogen bonding between the peptides and nucleic acids, dehydration and dissociation of the phosphates in the buffer solution were apparently observed. Thus, the interactions were too complex for calculation of the thermodynamical parameters. However, it was observed that both of the peptides selectively interacted with the RNA/RNA duplexes. In contrast, only Agb$_8$ (**5**) interacted with both the RNA/RNA and DNA/DNA duplexes. This result may be due to the fact that, in Agb$_8$, the side chain is long and sufficiently flexible to interact with the phosphate groups of both the RNA/RNA and DNA/DNA duplexes. In comparison with the amino-substituted Dap$_8$, the guanidine-substituted Dab$_8$ (**5**) had larger endothermic interactions.

Inhibition assays were carried out to clarify the peptide-binding sites in the RNA duplexes. The peptides were titrated into a solution of the RNA duplexes in the presence of neomycin, which is known to bind to the major groove of RNA duplexes.[18] The inhibition of peptide–RNA binding by neomycin was different for the types of peptides (Fig. S10[17]). For Dab$_8$ (**2**), the exothermic interactions were selectively inhibited, while the endothermic interactions were still observed. On the other hand, for Agp$_8$ (**5**), both of the interactions were inhibited. These results suggest that the exothermic interactions are attributed to the binding of the peptides to the major groove of RNA/RNA duplexes and the endothermic interactions are related to the interactions with other sites.

3. Materials and methods

3.1. Peptide synthesis

Peptides were synthesized via a conventional solid-phase method by using the 9-fluorenylmethyloxycarbonyl (Fmoc) strategy.[19] The peptide chains were assembled on a Fmoc-NH-SAL-PEG resin by using Fmoc amino acid derivatives (5 equiv), *N*,*N*-diisopropylethylamine (DIPEA, 10 equiv), and 2-(1*H*-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 5 equiv) in dimethylformamide (DMF) for the coupling, and 25% piperidine/DMF for the removal of the Fmoc group. After coupling of the last amino acids, amino groups at the N-termini were protected with an acetyl (Ac) group using acetic anhydride (10 equiv). To cleave the peptide from the resin and remove the side chain protecting groups, the peptide resin was treated with trifluoroacetic acid (TFA)-triisopropylsilane-water, (95:2.5:2.5, v/v/v). Peptides in sat $NaHCO_{3aq}$ (200 μl) were added in one portion to a solution of the 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine[20] (10 equiv per amino groups) in dioxane (200 μl), and stirred overnight at rt, then concentrated in vacuo. To remove the protecting groups from the guanidino groups, the peptides were treated with TFA–triisopropylsilane–water (95:2.5:2.5, v/v/v). All peptides were purified with reverse-phase HPLC (0.05% TFA in water–acetonitrile). The peptides were successfully identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Compound **1**, TOF-MS *m*/*z* calcd for $[M+Na]^+$ 1048.07; Found 1047.60. Compound **2**, TOF-MS *m*/*z* calcd for $[M+H]^+$ 1138.31; Found 1137.22. Compound **3**, TOF-MS *m*/*z* calcd for $[M+Na]^+$ 1272.50; Found 1271.56. Compound **4**, TOF-MS *m*/*z* calcd for $[M+H]^+$ 1362.73; Found 1361.51. Compound **5**, TOF-MS *m*/*z* calcd for $[M+H]^+$ 1362.41; Found 1361.51. Compound **6**, TOF-MS *m*/*z* calcd for $[M+H]^+$ 1474.63; Found 1473.77. Compound **7**, TOF-MS *m*/*z* calcd for $[M+H]^+$ 1586.84; Found 1585.72. Compound **8**, TOF-MS *m*/*z* calcd for $[M+H]^+$ 1419.46; Found 1418.53. Compound **9**, TOF-MS *m*/*z* calcd for $[M+Na]^+$ 1476.52; Found 1476.00. Compound **10**, TOF-MS *m*/*z* calcd for $[M+H]^+$ 1533.57; Found 1533.07. Compound **11**, TOF-MS *m*/*z* calcd for $[M+H]^+$ 1504.57; Found 1504.21. Compound **12**, TOF-MS *m*/*z* calcd for $[M+H]^+$ 1556.64; Found 1555.68. Compound **13**, TOF-MS *m*/*z* calcd for $[M+Na]^+$ 1100.07; Found 1099.25. Compound **14**, TOF-MS *m*/*z*

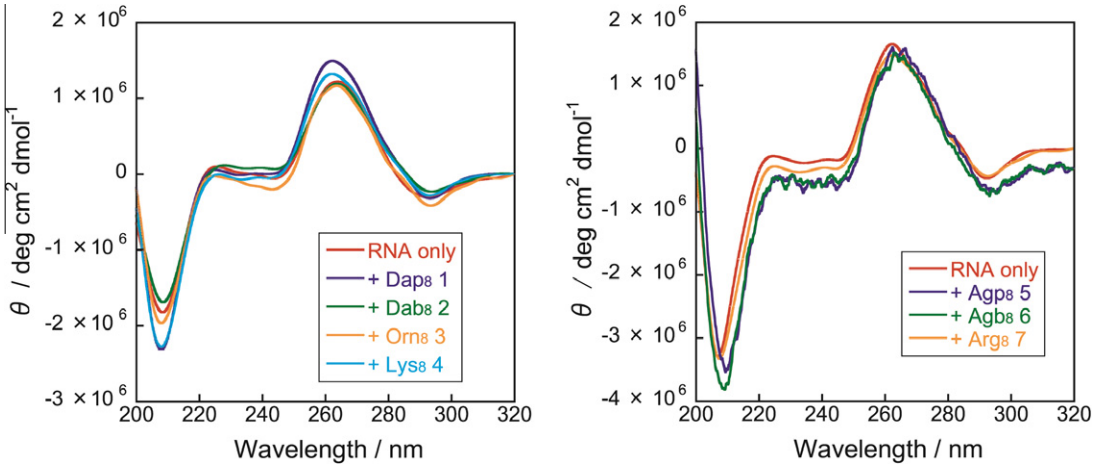


Figure 7. CD spectra of the RNA/RNA duplexes in the presence and absence of an equal amount of peptides **1**–**7** (at 20 °C, pH 7.0, 4 μM each of peptide and duplex).

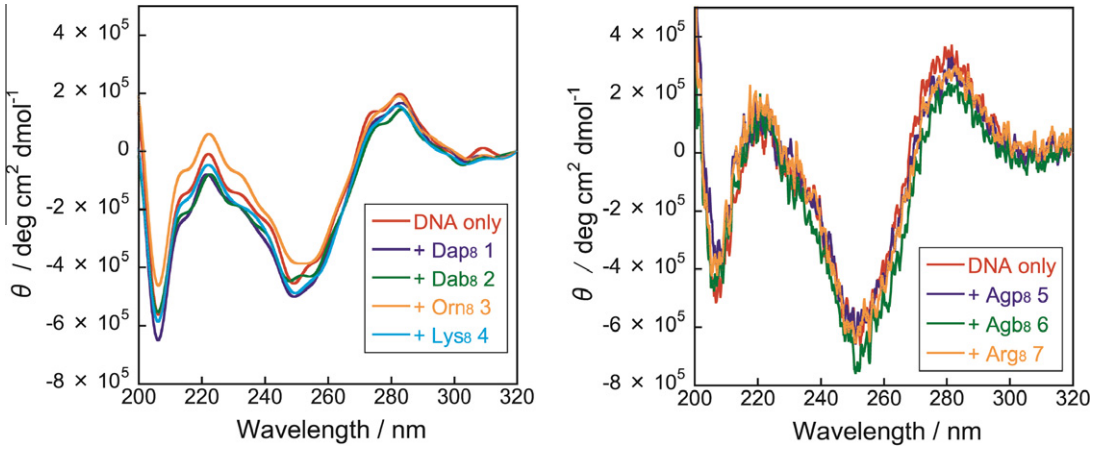


Figure 8. CD spectra of the DNA/DNA duplexes in the presence and absence of an equal amount of peptides **1–7** (at 20 °C, pH 7.0, 4 μM each of peptide and duplex).

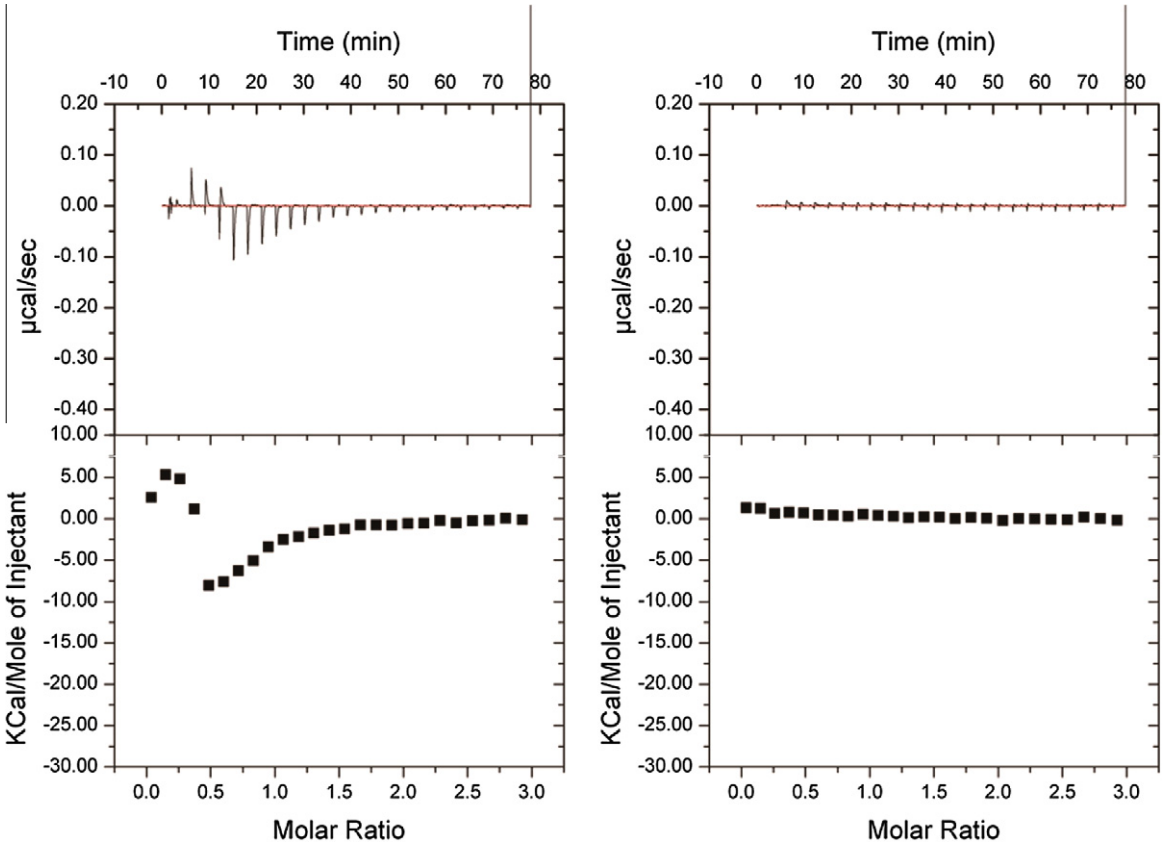


Figure 9. ITC profiles at 25 °C for titration of Dab_8 (**2**) into a solution of RNA/RNA duplex (left) and DNA/DNA duplex (right); each curve is the result of a 2.5 μl injection of 150 μM peptide. The duplex concentration was 10 μM in a 10 mM phosphate buffer with 100 mM NaCl at pH 7.0; corrected injection heat in the cases of RNA/RNA were plotted.

calcd for $[M+H]^+$ 1198.18; Found 1197.67. Compound **15**, TOF-MS *m*/*z* calcd for $[M+H]^+$ 1306.29; Found 1305.46.

3.2. Melting temperature (T_m) analysis

Absorbance versus temperature profile measurements were carried out in quartz cells with a 1 cm path length using an eight-sample cell changer. The variation in the UV absorbance with temperature was monitored at 260 nm. The temperature was scanned from 10 to 95 °C at a rate of 0.2 °C/min. The peptides were added after oligonucleotides were annealed. The samples were prepared as follows. The oligonucleotides were dissolved in a phosphate buffer (10 mM) containing NaCl (0.1 M) at pH 7.0. The solutions of oligonucleotides (4 μM) were first rapidly heated to 95 °C, left for 10 min, and then cooled to 10 °C at a rate of 1 °C/min. The equal amounts of peptides (final concn: 4 μM) were then added to the solution. The samples were left to equillbrate at the starting temperature for 30 min, the dissociation of the duplex was observed by heating the solution to 95 °C at a rate of 0.2 °C/min, and data points were collected at every 0.1 °C.

3.3. CD spectroscopy

All CD spectra were recorded at 20 °C. The following instrument settings were used: resolution, 0.1 nm; sensitivity, 10 mdeg; response, 4 s; speed, 10 nm/min; accumulation, 6.

3.4. Conditions for ITC experiments

The peptides and nucleic acid duplexes were dissolved in a 10 mM phosphate buffer containing 100 mM NaCl at pH 7.0. The peptide solutions (150 μM) were titrated into the nucleic acid duplex solutions (10 μM) at 25 °C. Each titration of peptide solution consisted of a preliminary 0.5 μl injection followed by 24 subsequent 1.5 μl additions, which were performed over 3 s periods at 120 s intervals. In the inhibition assays, the peptide solutions were titrated into the nucleic acid solution in the presence of 100 μM neomycin under the same conditions as described above.

4. Conclusion

We have synthesized a series of cationic oligopeptides by systematically changing the position of the cationic groups. On the basis of UV-melting analysis, CD spectrometry and ITC measurements, these cationic oligopeptides showed different tendencies for the stabilization of nucleic acid duplexes. Peptides with amino groups stabilized only RNA duplexes, while peptides with guanidino groups stabilized both RNA and DNA duplexes. In particular, Dab_8 (**2**) and Agp_8 (**5**) showed the highest T_m values among the series of peptides with the same cationic groups but different side chain length. These results suggest that the distance between the cationic groups, such as in Dab_8 (**2**) and Agp_8 (**5**), are well fitted to the distance between the phosphate groups in the major groove of RNA duplexes. Furthermore, peptides with alternate arrangements and those containing flexible amino acids did not stabilize the RNA duplexes. These results indicate that at least two consecutive sequences of Agp are necessary for effective binding of cationic oligopeptides to RNA duplexes. Therefore, given their unique properties, Dab_8 (**2**) and Agp_8 (**5**) will be useful as stabilizers of dsRNA-based nucleic acid drugs or new materials for their DDS.

Acknowledgments

We thank Professor Kohei Tsumoto (University of Tokyo) and Dr. Satoru Nagatoishi (University of Tokyo) for the ITC measurements and helpful discussions. This work was finally supported by KAKENHI and CREST, the Japan Science and Technology Agency.

Supplementary data

Supplementary data (data include CD spectra, UV melting profiles, and ITC profiles) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.01.053.

References and notes

1. Fire, A.; Xu, S.; Montgomery, M. K.; Kostas, S. A.; Driver, S. E.; Mello, C. C. *Nature* **1998**, *391*, 806.
2. Watts, J. K.; Deleavey, G. F.; Damha, M. J. *Drug Discovery Today* **2008**, *13*, 842.
3. (a) Manoharan, M. *Biochim. Biophys. Acta* **1999**, *1489*, 117; (b) Cook, P. D. *Nucleosides Nucleotides* **1999**, *18*, 1141.
4. Eguchi, A.; Meade, B. R.; Chang, Y. C.; Fredrickson, C. T.; Williert, K.; Puri, N.; Dowdy, S. F. *Nat. Biotechnol.* **2009**, *27*, 567.
5. Prakash, T. P.; Allerson, C. R.; Vickers, T. A.; Sioufi, N.; Jarres, R.; Baker, B. F.; Swayze, E. E.; Griffey, R. H.; Bhat, B. *J. Med. Chem.* **2005**, *48*, 4247.
6. Francois, B.; Russell, R.; Murray, J. *Nucleic Acids Res.* **2005**, *33*, 5677.
7. Maris, C.; Dominguez, C.; Allain, F. H. *FEBS J.* **2005**, *272*, 2118.
8. (a) Nurtola, M.; Zaramella, S.; Yeheskiely, E.; Strömberg, R. *ChemBioChem* **2010**, *11*, 2606; (b) Wu, C. H.; Chen, Y. P.; Mou, C. Y.; Cheng, R. P. *Amino Acids* **2012**. http://dx.doi.org/10.1007/s00726-012-1357-0.
9. Iwata, R.; Sudo, M.; Nagafuji, K.; Wada, T. *J. Org. Chem.* **2011**, *76*, 5895.
10. Lemberg, M. K.; Martoglio, B. *Mol. Cell* **2002**, *10*, 735.
11. Futaki, S.; Suzuki, T.; Ohashi, W.; Yagami, T.; Tanaka, S.; Ueda, K.; Sugiura, Y. *J. Biol. Chem.* **2001**, *276*, 5836.
12. Kim, S. W.; Kim, N. Y.; Choi, Y. B.; Yang, J. M.; Shin, S. *J. Controlled Release* **2010**, *143*, 334.
13. Russell, A. L.; Williams, B. C.; Spuches, A.; Klapper, D.; Srouji, A. H.; Hicks, R. P. *Bioorg. Med. Chem.* **2012**, *20*, 1723.
14. Jones, S.; Daley, D. T.; Luscombe, N. M.; Berman, H. M.; Thornton, J. M. *Nucleic Acids Res.* **2001**, *29*, 943.
15. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, G.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *143*, 334.
16. Ura, Y.; Leman, J.; Orgel, L. E.; Ghadiri, M. R. *Science* **2009**, *325*, 73.
17. See the Supplementary data.
18. Varani, L.; Spillantini, M. G.; Goedert, M.; Varani, G. *Nucleic Acids Res.* **2000**, *28*, 710.
19. *Fmoc Solid Phase Peptide Synthesis*; Chan, W. C., White, P. D., Eds.; Oxford University Press: New York, 2000. pp 42–76.
20. Xiao, S.; Fu, N.; Peckham, K.; Smith, B. D. *Org. Lett.* **2010**, *12*, 140.