Incorporation of 2′-*O*-Methyl-2-thiouridine into Oligoribonucleotides Induced Stable A-form Structure

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The conformational pre-organization of oligonucleotides containing two 2′-*O*-methyl-2-thiouridines (s^2Um) was studied. Melting temperature (T_m) and CD spectrum analysis of single strands and duplexes revealed that the s^2Um incorporation leads to a stable A-form structure.

2-Thiouridine (s^2U) and its derivatives have been discovered as modified nucleosides from tRNAs.[1,2] It is known that the sugar pucker of s^2U is a rigid N-type (C3′-*endo*) conformation because of the steric repulsion between the 2-thiocarbonyl groups and the 2′-hydroxyl group.[3,4] Based on such rigid sugar conformation, oligoribonucleotides containing s^2U and its derivatives form stable RNA–RNA duplexes.[5–9] Moreover, it was shown by X-ray analysis that poly-2-thiouridylic acid forms a stable A-form-like helix.[10] The extreme stability of poly-2-thiouridylic acid was due to the stacking effect between the 2-thiocarbonyl group of an upstream 2-thiouracil base and the 1-N nitrogen atom of a downstream pyrimidine ring. Therefore, the RNA duplex stability is expected to increase, particularly when consecutive s^2U base sequences are incorporated into oligoribonucleotides. Previously, we reported the synthesis of oligoribonucleotides incorporating an s^2Um and their properties.[8,9] s^2Um also has rigid C3′-*endo* conformation capable of stabilization of RNA duplexes. Accordingly, s^2Um could be regarded as a good s^2U analog. In this paper, we report the effect of consecutively incorporated s^2Um sequences on the RNA–RNA duplex stability which was analyzed by use of CD and T_m experiments.

s^2Um and its phosphoramidite building block were prepared by the procedures previously reported.[8] The s^2Um-containing oligoribonucleotides **2**–**4** were synthesized by use of phosphoramidites with PAC (phenoxyacetyl) for nucleobase protection and standard coupling conditions except that the iodine oxidation was replaced by *tert*-butyl hydroperoxide oxidation.[11] Release, deprotection, and desilylation of each oligoribonucleotide were carried out by successive treatments with aqueous ammonia–EtOH (4:1 = v/v) and 1 M tetrabutylammonium fluoride in THF. The fully deprotected RNA oligomers were purified by anion-exchange-HPLC. The oligoribonucleotides thus obtained were characterized by MALDI-TOF mass spectrometry. The sequence of oligoribonucleotides and RNA–RNA duplexes used in this study are shown in Table 1.

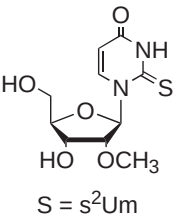
In order to study the detailed structure of RNA duplexes of **1**/**5**, **3**/**5**, and **4**/**5** formed between s^2Um-containing RNAs and their complementary RNA strands, these CD spectra were measured in 10 mM sodium phosphate buffer (pH 7.0) containing 150 mM NaCl at 5 °C. The results are as shown in Figure 1.

Interestingly, the RNA–RNA duplex **3**/**5** with a non-consecutive s^2Um sequence gave a characteristic CD spectrum. The intensity of the positive Cotton effect at around 260 nm was the strongest and sharpest in comparison with the other RNA duplexes. In general, the strong and sharp Cotton effect at around 260 nm is regarded as an indicator for RNA–RNA duplex formation. This result indicated that the duplex **3**/**5** forms a more rigid RNA–RNA duplex than the duplex **4**/**5** with a consecutive s^2Um sequence (XXX = USS line). Although we expected that the duplex **4**/**5** would form a more rigid and stable RNA duplex because of its rigid sugar pucker continuation and the strong stacking effect would lead to predominance of the A-form structure, the result obtained was absolutely unexpected. In order to clarify this intriguing result, we measured the CD spectra of the single RNA strands **2**–**4** containing s^2Um assuming the conformational fixation at the single strand level that could affect the conformation of the duplex through the so-called pre-organization mechanism. The CD spectra of **1**–**4** are shown in Figure 2.

As the result, the single strand **3** with a non-consecutive s^2Um sequence gave a unique CD profile having sharp and strong Cotton effect at around 260 nm which looks like a typical A-type RNA–RNA duplex. This result indicated that the non-consecutive s^2Um sequence of **3** could significantly contribute

Table 1. Sequence of oligoribonucleotides and RNA–RNA duplexes used in this study

1	rCGUUUUUGC
2	rCGUUSUUGC
3	rCGUSUSUGC
4	rCGUUSSUGC
5	rGCAAAAACG
1/5	rCGUUUUUGC / rGCAAAAACG
3/5	rCGUSUSUGC / rGCAAAAACG
4/5	rCGUUSSUGC / rGCAAAAACG



S = s^2Um

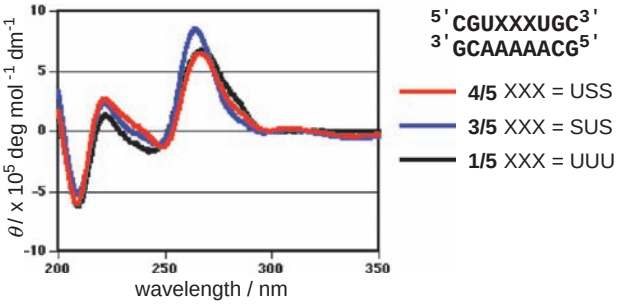


Figure 1. CD spectra of RNA–RNA duplexes containing s^2Um.

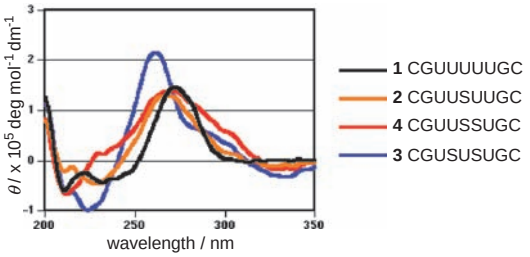


Figure 2. CD spectra of RNA single strands containing s^2Um.

to induction of an A-type form even in the single strand. This result suggested that the eminent unique A-type duplex structure of the duplex **3**/**5** shown in Figure 1 would be due mainly to the inherent conformational property of the single strand **3**. If this hypothesis is true, the melting temperature (T_m) of the duplex **3**/**5** will give a higher T_m value than those of the other duplexes because of the entropically favorable pre-organization effect originated from the rigidity of the single strand **3**.

To ascertain this hypothesis, we examined the melting temperature of the RNA–RNA duplexes. The UV melting curves of the duplexes were measured in 10 mM sodium phosphate buffer (pH 7.0) containing 150 mM NaCl. These results are shown in Table 2.

Table 2. Melting temperature (T_m Value, °C) of RNA–RNA duplexes containing s^2Um

CGUXXXUGC / GCAAAAACG	T_m value (ΔT_m)[a]	
1/**5** XXX = UUU (control)	36.7	
2/**5** XXX = USU	42.2 (+5.5)	
3/**5** XXX = SUS	48.3 (+11.6)	$\Delta\Delta T_m$ (+1.3)
4/**5** XXX = USS	47.0 (+10.3)	

[a] ΔT_m = (T_m value of a modified duplex) − (that of a wild-type duplex).

Consequently, the duplex **3**/**5** showed a significantly higher T_m value of 48.3 °C than that (47.0 °C) of the duplex **4**/**5**. More interestingly, the ΔT_m value (5.8 °C) per one modification of the duplex **3**/**5** was slightly higher than that of the duplex **2**/**5** (5.5 °C). On the other hand, the ΔT_m value (5.2 °C) per one modification of the duplex **4**/**5** was slightly lower than that of the duplex **2**/**5**. These results indicated that the duplex **3**/**5** forms a more stable duplex than the duplex **4**/**5**. It was previously shown by ^{1}H NMR analysis of a dimer of s^2UmpU that the s^2Um induced the C3′-*endo* conformation of the 3′-downstream uridine residue.[8] Because of this unique effect, it is likely that the single strand **3** can have a local A-type structure of (s^2Umps^2UmpU) having four continuous rigid C3′-*endo* sugar moieties. Similarly, the single strand **4** could have a three continuous rigid sugar sequence (s^2Umps^2UmpU). This difference between the single strands **3** and **4** might be the reason why the duplex **3**/**5** showed a higher T_m value. Because the single strand **3** has an additional rigid C3′-*endo* nucleotide unit and the conformation pre-organization effect is larger than in the single strand **4**, the duplex **3**/**5** could form a more stable A-form duplex. Namely, 2-thiouridine derivatives might induce C3′-*endo* conformation of a nucleoside at the 3′-downstream position, and the high thermal stability of poly-2-thiouridine helix may be due to this effect.

Similarly, such a neighboring nucleoside participation effect (long-range cooperative effect) was also reported in the case of oligonucleotides incorporating 5-(propyn-1-yl)pyrimidine derivatives by Turner et al.[12] The consecutive incorporation of 5-(propyn-1-yl)pyrimidine units resulted in extreme enhancement of the thermal stability of modified oligonucleotides. The stacked propynyl group stabilized helix geometry, while the unstacked propynyl group might disturb helix geometry.

We have revealed the unique properties of oligoribonucleotides incorporating two s^2Um units. The CD and T_m experiments showed that the non-continuous s^2Um incorporation stabilized the RNA–RNA duplex form more than the continuous s^2Um incorporation. This interesting phenomenon could be explained by the previous observation of the pre-organization that the s^2Um incorporation induced the C3′-*endo* conformation of the 3′-downstream nucleotide.

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