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Triplex forming ability of oligonucleotides containing 2′-*O*-methyl-2-thiouridine or 2-thiothymidine

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Abstract—The triplex forming ability of oligonucleotides containing 2′-*O*-methyl-2-thiouridine (s^2Um) and 2-thiothymidine (s^2T) was studied. The UV melting experiments revealed that triplex forming oligonucleotides (TFOs) containing both s^2Um or s^2T stabilized significantly parallel triplexes. The main reason for stabilization of triplexes was due to the stacking effect of the 2-thiocarbonyl group. Moreover, it turned out that these modified TFOs had a high selectivity in recognition of a matched Hoogsteen base from a mismatched one.

Many modified nucleosides have been tested to enhance the stability of triplexes.[1,2] These studies revealed that RNA incorporating triplex forming olignucleotides (TFOs), 2′-*O*-methylated RNA or BNA (LNA) stabilized parallel triplex structures.[2–4] These results indicate that the C3′-*endo* conformation of nucleotide blocks was an important factor for stabilization of parallel triplex structures.

On the other hand, it is known that the 2-thionation of the uracil base leads to stabilization of the C*3′-endo* conformation of the ribose moiety because of the steric repulsion between the 2-thiocarbonyl group and the 2′-hydroxyl group.[5,6] Therefore, oligonucleotides containing 2-thiouridine (s^2U) derivatives form stable RNA duplexes with the complementary RNAs.[7–10] Moreover, it was reported on the basis of X-ray analysis that poly-2-thiouridylic acids form extremely stable A-form helixes. The enhanced rigidity of poly-2-thiouridylic acids can be explained in terms of the strong stacking effect of the 2-thiocarbonyl group on the 5′-upstream 2-thiouracil base and the 1-*N* nitrogen atom of the 3′-downstream pyrimidine ring.[11,12] This strong stacking effect might be also contributed to stabilization of A-form RNA duplexes.

Since s^2U derivatives have rigid C*3′-endo* conformations and strong stacking ability, oligonucleotides containing these modified nucleosides can form not only stable duplexes but also more stable triplexes. Molecular modeling of a parallel triplex containing a s^2T base in the third strand suggested that the 2-thiocarbonyl group of the 5′-upstream s^2T base could interact with the 1-*N* nitrogen atom of the 3′-downstream pyrimidine ring (Fig. 1). This result implied that the 2-thiocarbonyl moieties also could exhibit strong stacking effects in

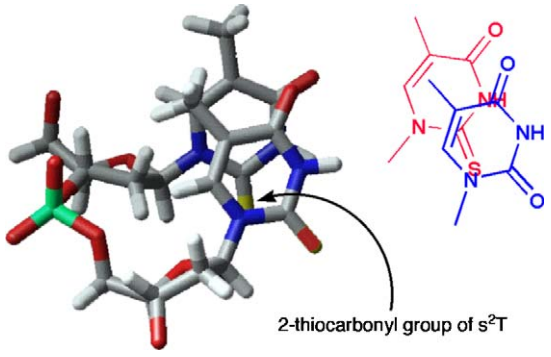

Figure 1. A part of s^2TpT structure of triplex forming oligonucleotide in triplex structure.

Keywords: Triplex forming oligonucleotides; Antigene strategy; 2-Thiouridine; 2-Thiothymidine; Hybridization ability; Hoogsteen base pair.
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TFOs. In this study, we evaluated the triplex forming ability of TFOs containing 2′-*O*-methyl-2-thiouridine (s^2Um) and 2-thiothymidine (s^2T).

It is known that oligonucleotides containing s^2U derivatives react with iodine that was used as an oxidizing reagent of phosphite intermediates during the oligonucleotide synthetic cycle.[13,14] However, in our recent study, we found that oligonucleotides containing s^2U derivatives could be synthesized by use of standard phosphoramidite chemistry without changing this oxidizing reagent. Namely, the use of iodine at a lower concentration of 0.02 M in pyridine-THF-H_2O resulted in no formation of byproducts arising from the side reactions. Therefore, we synthesized TFOs containing s^2Um or s^2T by use of standard phosphoramidite chemistry. The synthesis of the s^2Um phosphoramidite derivative was now established in our laboratory and the s^2T phosphoramidite building block is now commercially available.[10,15] The modified TFOs were successfully obtained in good yields (ca. 20–40%).

UV melting experiments were carried out with hairpin DNA that was composed of a 16 base pair stem region and a four base internal loop region, and TFOs were hybridized with their stem part. The sequences of TFOs and hairpin DNA used in this study are shown in Figure 2.

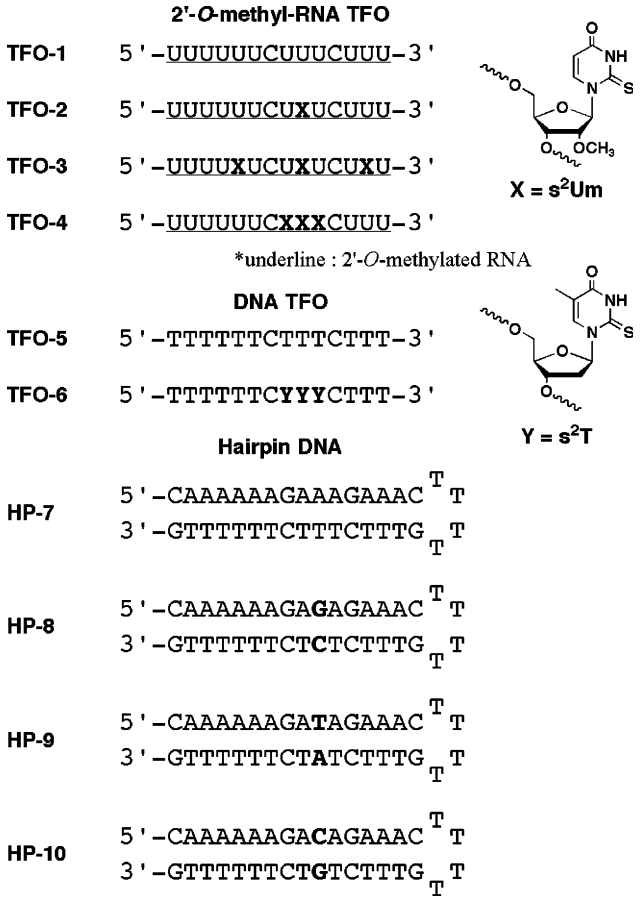

Figure 2. Sequence of triplex forming oligonucleotides and hairpin DNA used in this study.

These TFOs consist of uracil or thymine bases mostly because the cytosine base requires protonation of the 3-position so that an increase in the number of the cytosine base results in more destabilization of triplexes under the neutral conditions. Taking into account the relatively unstable property of triplexes, we carried out UV melting experiments under moderate salt conditions [10 mM sodium cacodylate buffer (pH 7.0) containing 500 mM NaCl and 10 mM $MgCl_2$] such that T_m values could be expected at temperatures with the range from 30 to 50 °C. In spite of the possibility that the cytosine base in **TFO-1** or **TFO-5** does not form a Hoogsteen base pair, the UV melting profiles and T_m values of unmodified triplexes **TFO-1/HP-7** and **TFO-5/HP-7** were successfully obtained at around 30 °C under neutral conditions. The results obtained by the T_m experiments of the modified triplexes are shown in Tables 1 and 2.

In the case of 2′-*O*-methyl-RNA **TFO-2** having a single point modification, the T_m value of the triplex was higher than the unmodified **TFO-1** (ΔT_m = +6.9 °C). Moreover, 2′-*O*-methyl-RNA **TFO-3**,**4** having three modification points formed more stable triplexes. On the other hand, the DNA **TFO-6** containing three s^2T moieties also showed a high T_m value, but the ΔT_m value was slightly lower than that obtained in the case of the s^2Um-containing 2′-*O*-methyl-RNA **TFO-4**. Theoretical studies on the effect of the modified base pair A-s^2U on

Table 1. Melting temperature (T_m value, °C) analysis of triplex containing s^2Um and s^2T

TFO/hairpin	T_m	ΔT_m	ΔT_m/mod[a]
2′-*O*-Methyl-RNA TFO			
TFO-1/HP-7 (unmodified)	33.0		
TFO-2/HP-7	39.9	+6.9	+6.9
TFO-3/HP-7	49.7	+16.7	+5.6
TFO-4/HP-7	51.1	+18.1	+6.0
DNA TFO			
TFO-5/HP-7 (unmodified)	28.2		
TFO-6/HP-7	42.6	+14.4	+4.8

Conditions: 10 mM sodium cacodylate buffer (pH 7.0), 500 mM NaCl, 10 mM $MgCl_2$, and 2.0 μM triplex.
[a] ΔT_m/mod value were ΔT_m/one modification.

Table 2. Melting temperature (T_m value, °C) analysis of triplex containing mismatch Hoogsteen-pairing site

TFO/hairpin	T_m	ΔT_m
Unmodified 2′-*O*-Methyl-RNA TFO		
TFO-1/HP-7 (Um•A:T)	33.0	
TFO-1/HP-8 (Um•G:C)	16.9	−16.1
TFO-1/HP-9 (Um•T:A)	16.0	−17.0
TFO-1/HP-10 (Um•C:G)	15.0	−18.0
2′-*O*-Methyl-RNA TFO containing s^2Um		
TFO-2/HP-7 (s^2Um•A:T)	39.9	
TFO-2/HP-8 (s^2Um•G:C)	16.6	−23.3
TFO-2/HP-9 (s^2Um•T:A)	15.0	−24.9
TFO-2/HP-10 (s^2Um•C:G)	15.0	−24.9

Conditions: 10 mM sodium cacodylate buffer (pH 7.0), 500 mM NaCl, 10 mM $MgCl_2$, and 2.0 μM triplex.

the hydrogen bond energy have been reported. These results suggest that the influence of 2-thionation of the uracil base does not affect significantly the hydrogen bonding ability. Based on these results, it seems that the main reason for enhancement of the triplex stability was due to the stacking effect of the 2-thiocarbonyl moiety.[16,17] It is likely that the sugar pucker of TFOs containing s^2Um becomes a rigid *C3′-endo* conformation so that higher T_m values were observed.

In the case of the hairpin DNAs **HP-8**–**10** having mismatched Hoogsteen-pairing sites, both of the unmodified **TFO-1** and the modified **TFO-2** show almost the same T_m values (Table 2). These results indicated that the modification site did not form a stable Hoogsteen base pair. Moreover, it can be assumed that mismatched Hoogsteen base pair of TFO induced the geometry change of the triplex structure and these conformational changes synchronized the stacking effects of the 2-thiocarbonyl group.

It was concluded that incorporation of s^2U derivatives into oligonucleotides is effective for stabilization of triplexes. The stabilization of triplex formation is mainly due to the strong stacking effects of the 2-thiocarbonyl group. Melting temperature analysis of mismatched Hoogsteen base pairs revealed that incorporation of 2-thiocarbonyl moieties in TFO did not reduce Hoogsteen base pair recognition abilities. Such properties are favorable for antigene strategy. More detailed studies of the mechanism associated with the stabilization of triplexes with s^2Um or s^2T are now under progress.

Acknowledgments

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