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Selective formation of non-natural type triplexes containing TA interrupting sites with the TFO incorporating W-shape nucleic acid (WNA) analogs

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ABSTRACT

Novel nucleoside analogs have been desired for selective formation of non-natural type triplexes containing TA or CG interrupting sites. We have previously reported that the W-shaped nucleic acid (WNA) would be a useful skeleton to develop new base analog for the formation of non-natural triplexes of antiparallel motif. In this study, we have found that triplex forming oligonucleotide (TFO) incorporating the new WNA analog, WNA-βT, formed more stable triplex than natural triplex with high selectivity to the TA site. It is also noted that the TFO containing WNA-βT did not form aggregates at the physiological condition of K^+.

INTRODUCTION

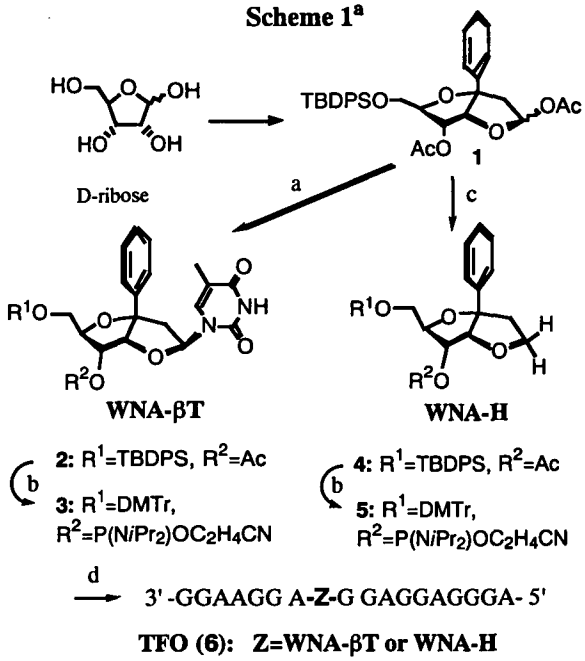
Triplexes formed between the oligonucleotide and the duplex DNA within the major groove have been investigated as a tool for modulation of gene expression in a sequence specific manner at the level of transcription.[1] However, the duplex sequence for triplex formation is limited to the homopurine-homopyrimidine stretch, in which TA or CG base pairs interrupt triplex formation. Despite of a number of studies, general solution has not appeared for this problem.[2]

Recently, we have designed the new base analog (WNA, W-shape nucleic acid) bearing a base part and a benzene ring on the bicyclic skeleton, and have demonstrated that the WNA-7βG-incorporating oligodeoxynucleotide can form triplex with specificity to the duplex with a TA interrupting site.[3] It was expected that the benzene ring might contribute stability of triplexes with stacking interaction and that a guanine might form hydrogen bonds to the target base pair. As the bicyclic skeleton seemed to be useful as a new scaffold for development of new WNA analogs for better recognition properties of TA and CG interrupting sites, we synthesized a number of WNA analogs with different base structure and stereochemistry of *N*-glycoside linkage. In this paper, we wish to report that WNA-βT having thymine as a base unit with β-stereochemistry exhibited higher selectivity as well as stability toward a TA interrupting site in anti-parallel triplex formation.

RESULTS AND DISCUSSION

Chemistry

Scheme 1[a]



[a] a) thymine, HMDS, Me_3SiCl, $SnCl_4$, CH_3CN, r.t. to 50°C, b) (i) TBAF, THF, 2. 0.2 M NaOHaq, MeOH, (ii) DMTrCl, pyridine, (iii) iPr_2NEt, CH_2Cl_2, $ClP(NiPr_2)OC_2H_4CN$, (c) Et_3SiH and TMSOTf, (d) (i) DNA synthesis, (ii) 28% NH_4OH, (iii) HPLC purification, (iv) 10% aq. AcOH.

The key bicyclic intermediate **1** was prepared from D-ribose in 10 steps as previously reported (Scheme 1).[3] Treatment of **1** with thymine in the presence of HMDS, TMSCl and Sn_4Cl in acetonitrile[4] furnished *N*-glycosidation to produce a mixture of isomers (**2**) in a ratio of α: β=37:42. The two isomers were separated and its stereochemistry was determined by ^{1}H-COSY and NOESY spectrum. Each isomer was transformed to the corresponding amidite precursor (**3**). The abasic-type WNA-H (**4**) was prepared from **1** *via* reduction with Et_3SiH and TMSOTf, followed by the transformation to the amidite precursor (**5**). The amidite precursors were

incorporated into TFO (**6**) by automated DNA synthesizer. The synthesized TFO was cleaved from the resin with 28% NH_4OH, and purified by HPLC. The structures of TFOs were confirmed by MALD-TOF MASS measurement.

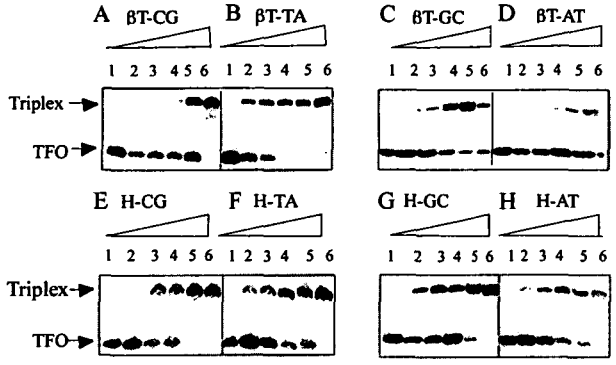


Figure 1. Determination of triplex formations and binding constants. Triplex formation was done for 12 h at 22°C in the buffer containing 20 mM Tris-HCl, 5 mM $MgCl_2$, 2.5 mM spermidine and 10% sucrose at pH 7.5. Electrophoresis was done at 10°C with 15% non-denatured polyacrylamide gel. A-H: 10 nM TFO containing ^{32}P-labeled one as the tracer used. The concentration of duplex was increased from lane 1 to 6 (nM); 0, 4, 8, 10, 40, 100. The combination Z-XY is shown such as βT-CG.

Table 1. Binding constants (*Ks*) for natural type (Z=G), WNA-βT and WNA-H.

Z	(X,Y) G,C	A,T	C,G	T,A
G	0.096	0.01	0.01	0.007
WNA-βT	0.09	< 0.001	0.018	0.31
WNA-H	0.19	0.045	0.20	0.068

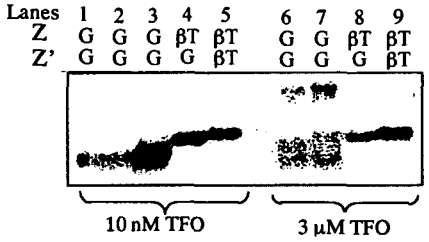
*Bands of Figure 2 were quantified and subjected to the following equation to produce binding constants.

$$Ks = \frac{[\text{triplex}]}{[\text{ssTFO}]x[\text{duplex}]}$$

Evaluation of triplex formation

The triplex-forming ability of TFOs incorporating the new WNA analogs (WNA-βT or WNA-H) was analyzed by gel shift assay with 15% non-denatured polyacrylamide gel at 10°C with the use of the ^{32}P-labeled TFO as tracer (Figure 1). The triplex was identified as the less moving band relative to the single stranded TFO. In the combination of WNA-βT:TA (Figure 1B), triplex formation was observed at 4 nM duplex (lane 3) and almost all TFO was converted to the triplex at 10 nM duplex concentration (lane 4). On the other hand, the other combination of WNA-βT with CG (A), GC (C) and AT (D) produced the triplexes only at high concentration of the duplex. These results clearly indicated that WNA-βT is selective to a TA interrupting site. It is interesting that the TFO incorporating WNA-H (Figure 1, E-H) showed non-selective binding to all four duplex targets, suggesting that WNA-H would work as a universal base for triplex formation. Association constants of triplex formation were obtained by quantification of the results of the gel-shift assay (Table 1). The combination of WNA-βT:TA gave the largest *Ks* value, which is larger than that of the natural combination of G:GC. It is clearly suggested from comparison of *Ks* values in Table 1 that WNA-βT exhibits high selectivity by stabilization effect to a TA site together with destabilization effect to the other base pairs.

We further investigated aggregation behavior of TFOs, because purine-rich oligodeoxynucleotides are known to have tendency to form G-quadruplex or other disordered aggregation, especially in the presence of physiological concentration of potassium ion.[5] TFOs were incubated in buffer (pH 7.5) containing 150 mM KCl, then subjected to gel shift assay (Figure 2). The mobility of the natural TFO (**6**, Z=Z'=G) incubated in the absence of KCl is shown in lane 1. The natural TFO (**6**) did not show aggregation at the low concentration (10 nM, lane 2 and 3), whereas it formed slow-mobility bands at higher concentration (3 μM). In contrast, the TFOs containing WNA-βT did not indicate the formation of aggregation even at high concentration (lane 4,5 *vs* 8, 9). It should be noted that WNA-βT showed not only selective stabilization effect to a TA interrupting site but also inhibitory effect for aggregation in the presence of physiological concentration of KCl.



TFO 3' GGAAGG AZZ' GAGGAGGGA 5'

Figure 2. Gel shift assay to check aggregation of the TFOs. TFOs were incubated in the presense of 150 mM of KCl in the buffer containing 20 mM Tris-HCl buffer (pH 7.5), 5 mM magnesium chloride, 2.5 mM spermidine and 10% sucrose. Electrophoresis was done at 10°C with 15% non-denatured polyacrylamide gel. Lane 1, TFO (10 nM, X=G) was incubated in the absence of KCl. Non-labeled 5'-phosphate[6] TFO was added in lane 3 and 7, indicating that 5'-phosphate did not inhibit aggregation at high concentration.

CONCLUSION

We have demonstrated that WNA-βT is a promising candidate for the formation of non-natural type triplexes containing TA interrupting sites with high affinity. Furthermore it is suggested that the TFOs incorporating WNA-βT attain resistance to aggregation. Triplex formation toward the duplex with other sequences is now in progress.

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6. Prepared according to the method in Guzaev, A., Salo, H., Azhayev, A., Lonnberg, H., *Tetrahedron*, **51**, 9375-9384, (1995).