Design and evaluation of novel nucleoside analogs (WNA) for specific formation of non-natural type triplexes containing a TA or CG interrupting site

Yosuke Taniguchi¹, Yusuke Senko¹, Kelichi Kodama¹, Ayako Nakamura¹ and Shigeki Sasaki¹,²
¹Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan and ²CREST (JST)

ABSTRACT
In a search of suitable recognition molecules for the formation of stable non-natural triplex DNA, we have developed the new nucleoside analogs (WNA, W-shape nucleoside analog) bearing an aromatic ring, a recognition base and a bicyclic skeleton to hold them. A successful result with WNA-βT that recognizes a TA base pair selectively has suggested that the basic structure of the WNA is useful as a scaffold for search of other candidates for the formation of triplex containing a TA or a CG interrupting site. In this study, we have synthesized and investigated binding property of a variety of WNA candidates. As a result, we have determined new potential analog, WNA-βC for a CG interrupting site. It should be noted that the triplexes containing a WNA-βT:TA or a WNA-βC:CG base triplet have exhibited higher stability than the natural anti-parallel triplexes constructed of A:AT and G:GC triplets. The results that H-WNA-βT lacking a benzene ring did not show stabilization effect to any base pairs have clearly indicated that a benzene ring plays a key role in stability of triplexes.

INTRODUCTION
Triplex formation at any predetermined duplex DNA using the triplex forming oligonucleotides (TFOs) would become useful tools for modulation of gene expression in the genomic research.¹ In the anti-parallel triplex formed with the purine motif TFO, the TFO binds to the homopurine stretch of duplex DNA in a sequence specific manner based on two reverse Hoogsteen hydrogen bonds of G:GC and A:AT base triplets.² However, as pyrimidine bases have one hydrogen bonding site in the major groove, their interruption in the homopurine strand inhibit stable triplex formation. This problem has been remained unsolved despite of much effort during last decade.³

Recently, we have developed new bicyclic nucleoside analogs (WNA) for stabilization of triplexes including interrupting sites.⁴ The WNA was designed to have three structure components; an aromatic ring as a stacking part, a heterocyclic ring as a recognition part and a bicyclic skeleton to hold these components (Fig. 1). Previously, we have reported that WNA-βT is a selective base analog for a TA interrupting site.⁵ In our effort to search potential candidates for triplex formation, we have been continuing evaluation of a variety of WNA compounds with different recognition part. In this paper, we describe that WNA-βC (Figure 2) having cytosine as a recognition base with β-stereochemistry has exhibited selective stabilization toward a CG interrupting site.

RESULTS AND DISCUSSION

Figure 1. The Structure of WNA-βT and WNA-H.

The WNA compounds including WNA-βC were synthesized by a similar method as reported previously.⁴,⁶ and were incorporated into the TFOs with an automated DNA synthesizer by the conventional amidite chemistry. The compound (H-WNA-βT) lacking a benzene ring was prepared from D-ribose by multi-step synthesis including α-selective allylation⁷ using a trithylperchlorate. All the TFOs were purified by reverse-phase HPLC and their purity and structure were confirmed by MALDI-TOF MS.
measurements.

Figure 2. The Structure of WNA-βC and H-WNA-βT.

Table 1. Association constants (Ks) of Triplex (10^9) for natural type and WNA analogs in the presence of 5 mM MgCl2.

<table>
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<th>Z</th>
<th>TA</th>
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<th>CG</th>
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(a) Triplex formation was done for 12 h at 22°C in the buffer containing 20 mM Tris-HCl, 5 mM MgCl2, 2.5 mM spermidine and 10% sucrose at pH 7.5. 10 nM TFO containing 32P-labeled one as the tracer and different duplex concentrations ranging from 0 to 100 nM were used. Electrophoresis was done at 10°C with 15% non-denatured polyacrylamide gel, and radioactive bands corresponding to the single strand TFO and those in the triplex were quantified to give the association constants.

Evaluation of triplex formation

The triplex-forming ability of TFOs incorporating a new WNA analog was analyzed by gel shift assay with 15% non-denatured polyacrylamide gel at 10°C with use of the 32P-labeled TFO as the tracer. The triplex was identified as the less migration band relative to the single stranded TFO. In the combination of WNA-βC:CG, triplex formation was observed at 10 nM duplex for the 10 nM TFO, and almost all TFO was converted to the triplex at 40 nM duplex. On the other hand, the other combination of WNA-βC with GC, TA or AT needed higher concentration of the target duplex for triplex formation. Association constants (Ks) were obtained by quantification of the bands (Table 1), indicating that WNA-βC is selective to a CG interrupting site. From the comparison with the association constants of WNA-H, it turned out that destabilization effect of a cytosine unit of WNA-βC is less in the combination WNA-βC:CG than in the other combinations. It should be noted that stability of the triplexes including a WNA-βC:TA or a WNA-βC:CG combination is much higher than the natural ones containing a G:GC or a A:AT triplet (Table 1). As H-WNA-βT lacking a benzene ring did not produce effective stabilization to any base pairs, benzene ring apparently plays a major role in stabilization of triplexes.

CONCLUSION

We have demonstrated that WNA-βC is a selective base analog to a CG interrupting site to form stable non-natural type triplex. Although selectivity of WNA-βC to a CG pair seems to be insufficient, it turned out that we now have recognition units for all four base pairs. WNA derivatives would be the potential candidates to expand limitation of triplex code of anti-parallel triplexes.

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REFERENCES