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Effective strand invasion by ODN incorporating a new bicyclic nucleoside analogue (WNA)

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ABSTRACT

Efficient and specific targeting of DNA sequences by synthetic ligands is a major goal in chemical biology. Triplex-forming oligonucleotides (TFOs) are sequence-specific DNA-binding agents, but are limited to homopurine/homopyrimidine target sequences. We have previously reported two useful analogues (WNA: W-shaped nucleoside analogues), WNA-βT and WNA-βC, which recognize a TA and a CG interrupting site forming triplexes with high stability and selectivity, respectively. However, their ability to form triplexes depended on their neighbouring bases in the TFO. Subsequent studies have shown that the sequence-dependency of the WNA analogues, for the formation of triplexes, has been partially solved by use of a WNA analogue bearing a substituted aromatic ring. Investigations into the effects of the substituted aromatic ring of WNA derivatives on the stability of triplexes led to the discovery of strand invasion by the TFO incorporating the new WNA analogue to form a highly stable duplex.

INTRODUCTION

Efficient and specific targeting of DNA sequences by synthetic ligands is a major goal in chemical biology. Three approaches, triplex-forming oligonucleotides (TFOs), helix-invading peptide nucleic acids and minor groove binders, are available for this purpose, but there are unresolved limitations on base pair selectivity, target length and/or sequence specificity.

TFOs are sequence-specific DNA-binding agents and recognize DNA sequences by binding with hydrogen bonds between the bases in the third strand and those in the purine strand of the duplex.[1] However, the stable triplex formations are limited to homopurine/homopyrimidine target sequences and prevented by the presence of one pyrimidine base in the purine strand. This problem has not been fully solved.[2-4] Therefore, the search for nucleoside analogues that recognize the limitation sites in DNA and enable triplex formation at mixed-sequence target sites is needed for the utilization of TFOs as tools for genomic research, technology and therapy.

We have previously reported the novel nucleoside analogue WNA (W-shaped nucleoside analogues) with a bicyclic skeleton bearing an aromatic component for stacking and a nucleobase for Hoogsteen hydrogen bonds to form triplex.[5] Furthermore, we have developed WNA analogues, WNA-βT and WNA-βC, which can recognize a TA and a CG interrupting site to form triplexes with high stability and selectivity, respectively, but their ability to form triplexes depended on their neighbouring bases in the TFO.[6, 7] Recently, we reported that the sequence-dependency of the WNA analogues, for the formation of triplexes, has been partially solved by use of WNA analogues with a modified aromatic ring. The effect of these WNA derivatives on the stability of the triplex has been investigated.[8, 9] During these investigation the *o*Me-WNA-βT derivative (Figure 1) was found to exhibit an interesting characteristic when strand invasion of the target duplex was observed.

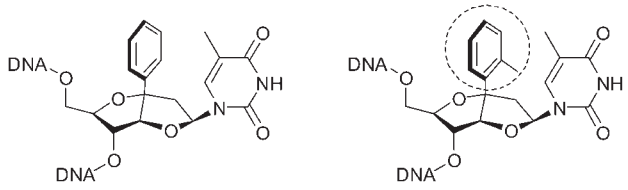

Figure 1. Structures of WNA-βT and *o*Me-WNA-βT

RESULTS AND DISCUSSION

Several WNA derivatives with a variety of aromatic ring components were synthesized from D-ribonolactone by a similar method as previously reported. The WNA-βT derivatives were converted to the corresponding amidite precursors, and incorporated into the TFOs by using an automated DNA synthesizer. The synthesized TFOs were purified by reverse-phase HPLC and treated with 10% aqueous acetic acid. The structure and purity of the TFOs were confirmed by MALDI-TOF MS measurement.

The triplex-forming ability of TFOs containing WNA analogues was evaluated by gel shift assay with 15% non-denatured polyacrylamide gel at 10°C using the ^{32}P-labeled TFOs as a tracer. The triplex was observed as the slower migrating band relative to the single stranded TFOs.

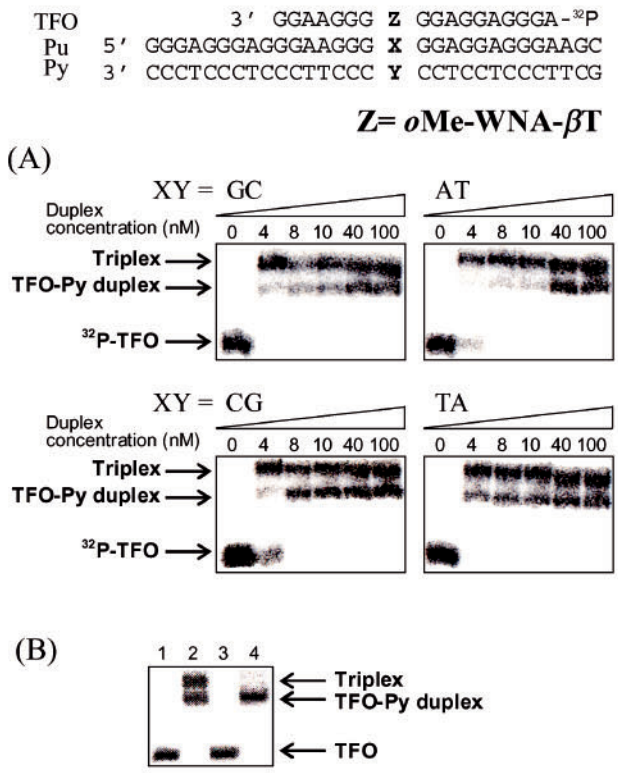

Figure 2. (A) Gel-shift assay for determination of triplex formation with TFO containing *o*Me-WNA-βT. Triplex formation was done for 12h at 22°C in a buffer containing 20 mM Tris-HCl, 5 mM $MgCl_2$, 2.5 mM spermidine and 10% sucrose at pH 7.5. Electrophoresis was done at 10°C with 15% non-denatured polyacrylamide gel. 10 nM TFO containing the ^{32}P-labeled one as the tracer and different target duplex concentrations raging from 0 to 100 nM were used. (B) Gel-shift assay for determination of the new complex formation with TFO containing *o*Me-WNA-βT under the same condition as shown in A. Lane 1, 10 nM labeled TFO only; lane 2, addition of 10 nM Pu-Py duplex; lane 3, addition of 10 nM Pu strand (X=T); lane 4, addition of 10 nM Py strand (Y=A).

Interestingly, the TFO containing *o*Me-WNA-βT formed two lower mobility bands in the gel-shift assay; the one corresponded to the triplex formation, and the other suggested the formation of a new complex. The triplex bands were formed almost equally when *o*Me-WNA-βT was paired opposite all four natural bases, indicating that *o*Me-WNA-βT displays a non-selective stabilization effect for triplex formation. In a similar gel-shift assay, addition of just the purine strand to the TFO did not result in the lower mobility band (Figure 2B, lane 3). In contrast, addition of just the pyrimidine strand to the TFO gave the lower mobility band (Figure 2B, lane 4) identical to the band observed with the TFO and the target Pu-Py duplex, suggesting the formation of a duplex between the TFO and the pyrimidine strand. Formation of the TFO-Py duplex was assumed to occur through a strand exchange reaction, therefore we compared the thermal stabilities of the TFO-Py duplex and the target Pu-Py duplex by measuring UV melting curves. The pyrimidine strands formed duplexes with the TFO with higher or similar stability to that of the fully-matched duplexes. The difference in thermal stability between these duplexes dose not fully explain the observed strand exchange reaction. The fact that the TFO-Py duplex band was larger than that of the triplex band in the gel-shift assay, after a short reaction period of 1 hour, suggests that the TFO-Py duplex is the kinetically favorable product and the triplex is the thermodynamically stable product. It should be noted that the *ortho*-methyl group inhibits free rotation of the benzene ring within WNA-βT. However, it is unclear how such a small structural change affects the strand exchange ability.

CONCLUSION

This study has shown that not only dose triplex formation occur but also a strand exchange reaction takes place with a TFO incorporating *o*Me-WNA-βT. Further studies are now ongoing to reveal the detailed mechanism of the strand exchange reaction.

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