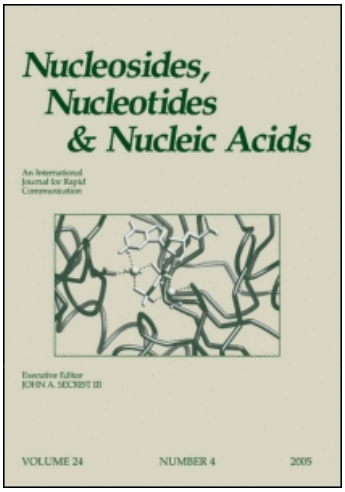

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2′,4′-BNA^{NC}: A NOVEL BRIDGED NUCLEIC ACID ANALOGUE WITH EXCELLENT HYBRIDIZING AND NUCLEASE RESISTANCE PROFILES

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□ *Oligonucleotides modified with 2′,4′-BNA^{NC} (N-H)/(N-Me) monomers exhibited excellent hybridizing and nuclease resistance properties. Duplex and triplex thermal stabilities were greatly enhanced by incorporating 2′,4′-BNA^{NC} (N-H) and (N-Me) monomers and nuclease resistance was tremendously higher than that of natural oligonucleotide.*

Keywords Nuclease resistance; stability; BNA; oligonucleotide

INTRODUCTION

Recently, regulation of gene expression by artificial nucleic acids has attracted a great deal of attention from both chemists and biologists.[1,2] Bridged Nucleic acids (BNAs)[3] are a recently developed class of artificial nucleic acids having profound applications in this area. Among the BNAs, 2′,4′-BNA[4] (also known as LNA)[5] (Figure 1) oligonucleotides, owing to their excellent hybridizing property and better nuclease resistance property than that of natural oligonucleotides, are the most promising and most widely used BNA oligonucleotides for various genomic technologies.[6,7]

As our understanding in the biological processes becomes sophisticated with the synthesized molecules, the demands on chemists increase to produce more radical molecules in order to control them. 2′,4′-BNA, having a five-membered bridged structure possessed excellent hybridizing ability to target strands. However, its nuclease resistance property, although fairly

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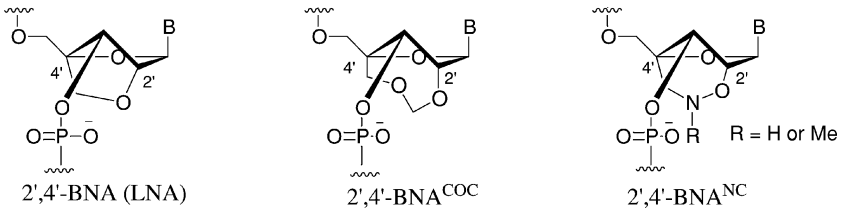

FIGURE 1 Structure of potent BNAs.

better than that of natural oligonucleotides, was not high enough. Considering this, we initially developed a nucleic acid analogue, 2′,4′-BNA^{COC} and found that this nucleic acid analogue provided excellent nuclease resistance property.[8] However, its hybridizing ability is lowered greatly, compared to that of 2′,4′-BNA. Therefore, as a continued effort to develop superior BNA analogues, we designed and synthesized a novel bridged nucleic acid analogue 2′,4′-BNA^{NC} with N-O bridged structure.[9] Two sets of oligonucleotides containing 2′,4′-BNA^{NC} (N-H) and (N-Me), respectively, were synthesized and their duplex- and triplex-forming abilities were examined and compared with those of natural oligonucleotides.

MATERIALS AND METHODS

Natural oligonucleotides (**DNA-1**: 5′-d(GCGTTTTTTGCT)-3′ and **DNA-2**: 5′-d(TTTTT^{m}CTTT^{m}CT^{m}CT^{m}CT)-3′) and oligonucleotides modified with 2′,4′-BNA^{NC} (N-H) and (N-Me) monomers (**ON-1**: 5′-d(GCGTT**X**TTTGCT)-3, **ON-2**: 5′-d(GCGTT**Y**TTTGCT)-3′, **ON-3**: 5′-d(GCG**X**T**X**T**X**TGCT)-3′, **ON-4**: 5′-d(GCG**Y**T**Y**T**Y**TGCT)-3′, **ON-5**: 5′-d(TTTTT^{m}CT**X**T^{m}CT^{m}CT^{m}CT)-3′, **ON-6**: 5′-d(TTTTT^{m}CT**Y**T^{m}CT^{m}CT^{m}CT)-3′; (**X** = 2′,4′-BNA^{NC} (N-H) thymine and **Y** = 2′,4′-BNA^{NC} (N-Me) thymine monomers, respectively; ^{m}C = 5-methylcytidine) were synthesized in an automated DNA synthesizer, purified and characterized by MALDI-TOF mass spectra. Target strands used for duplex and triplex forming experiments were 5′-r(AGCAAAAACGC)-3′ and 5′-d(GCTAAAAAGAAAGAGAGATCG)-3′/3′-d(CGATTTTTCTTTCTCTCTAGC)-5′, respectively. UV melting experiments were accomplished on a Beckman DU650 spectrometer equipped with a T_m measuring accessory. For the duplex-forming experiment, the particular oligonucleotide and target were dissolved in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM sodium chloride to give the final strand concentration of 4 μM. For the triplex-forming experiment, the particular oligonucleotide and target duplex were dissolved in 7 mM Na_2HPO_4 buffer (pH 7.0) containing 140 mM potassium chloride to give the final strand concentration of 1.5 μM. The temperature of the experiment was increased from 5 to 90°C with a constant rate of 0.5°C/minute. For the

TABLE 1 UV melting temperatures (T_m) of 2′,4′-BNA^{NC} modified oligonucleotides against a complementary ssRNA[a]

Oligonucleotides	T_m (°C)	ΔT_m (°C)	ΔT_m/mod.[b]
DNA-1	45	—	—
ON-1	51	+6.0	+6.0
ON-2	50	+5.0	+5.0
ON-3	64	+19	+6.3
ON-4	63	+18	+6.0

[a]Conditions: See Materials and Methods.
[b]ΔT_m/mod. = changes in melting temperature per single modification.

nuclease resistance experiment, a testing oligonucleotide (25 μg/ml) in 50 mM tris-HCl buffer (pH 8.0) containing 10 mM $MgCl_2$ was exposed to snake venom phosphodiesterase (SVPDE, Boehringer Mannheim) at 37°C.

RESULTS AND DISCUSSIONS

Duplex-forming ability of the natural oligonucleotide **DNA-1** and 2′,4′-BNA^{NC} modified oligonucleotides (**ON-1** to **ON-4**) was examined via T_m measurement and the results are summarized in Table 1. Against a complementary single-stranded RNA (ssRNA), T_m values of the oligonucleotides modified with a single 2′,4′-BNA^{NC} (N-H) and (N-Me) monomers (**ON-1** and **ON-2**, respectively) were increased by 6°C and 5°C, respectively. By incorporating three 2′,4′-BNA^{NC} monomers, T_m values further increased and the T_m values of **ON-3** and **ON-4** are 19°C and 18°C higher, respectively, than that of the natural oligonucleotide (**DNA-1**). These results show that duplex thermal stability is greatly enhanced by incorporating both the 2′,4′-BNA^{NC} (N-H) and (N-Me) monomers.

Triplex-forming ability at a physiological pH was evaluated against 21-bp double-stranded DNA (dsDNA). It was found that a single modification by the 2′,4′-BNA^{NC} (N-H) monomer (**ON-5**) increased the triplex stability by 11°C (Table 2). Triplex-forming ability of the 2′,4′-BNA^{NC} (N-Me) modified oligonucleotides **ON-6**, although a little lower than that of the 2′,4′-BNA^{NC} (N-H) oligonucleotide, is fairly higher than that of natural DNA (**DNA-2**). The lower triplex stability of 2′,4′-BNA^{NC} (N-Me) compared to 2′,4′-BNA^{NC}

TABLE 2 T_m Values of triplexes formed by 2′,4′-BNA^{NC} modified oligonucleotides[a]

Oligonucleotides	T_m (°C)	ΔT_m (°C)
DNA-2	33	—
ON-5	44	+11.0
ON-6	38	+5.0

[a]Conditions: See Materials and Methods.

(N-H) might be attributed to steric hindrance of the methyl group or lack of hydration.

Nuclease resistance property of 2′,4′-BNA^{NC} modified oligonucleotides was evaluated by exposing the oligonucleotides to SVPDE. While natural oligonucleotides decomposed completely within 5 minutes, 20% of 2′,4′-BNA^{NC} (N-H) and more than 60% of 2′,4′-BNA^{NC} (N-Me) oligonucleotides survived after 90 minutes. The nuclease resistance was also better than that of 2′,4′-BNA (LNA) oligonucleotide.

CONCLUSION

Modification of natural oligonucleotides by 2′,4′-BNA^{NC} (N-H) and (N-Me) greatly enhanced the duplex and triplex stability against ssRNA and dsDNA, respectively. The Nuclease resistance property of both 2′,4′-BNA^{NC} (N-H) and (N-Me) modified oligonucleotides was very much higher than that of natural oligonucleotide. In addition to the above exciting properties, the 2′,4′-BNA^{NC} (N-H) analogue offers a site (N-atom) on the bridged structure for further functionalization with various functional moieties (such as florescence, DNA cleavage activator) which would provide versatile applications in genomics.

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