© 2008 Oxford University Press
Symposium date: 8th September 2008

Nucleic Acids Symposium Series No. 52 **419–420**
doi:10.1093/nass/nrn213

Promotion of Triplex Formation by 2'-*O*,4'-*C*-Aminomethylene Bridged Nucleic Acid (2',4'-BNA^{NC}) Modification

Kiyomi Sasaki[1], S. M. Abdur Rahman[2], Satoshi Obika[2,3], Takeshi Imanishi[2], and Hidetaka Torigoe[1*]

[1]Department of Applied Chemistry, Faculty of Science, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601, Japan, [2]Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan, and [3]PRESTO, JST, Sanbancho Building, 5-Sanbancho, Chiyoda-ku, Tokyo 102-0075, Japan

ABSTRACT

We examined the effect of 2'-*O*,4'-*C*-aminomethylene bridged nucleic acid (2',4'-BNA^{NC}) backbone modification of triplex-forming oligonucleotide (TFO) on the pyrimidine motif triplex formation at neutral pH, a condition where pyrimidine motif triplexes are unstable. The melting temperature of the pyrimidine motif triplex at pH 6.8 with 2',4'-BNA^{NC} modified TFO was significantly higher than that observed with unmodified TFO. The 2',4'-BNA^{NC} modification of TFO increased the thermal stability of the pyrimidine motif triplex at neutral pH. The present results certainly support the idea that the 2',4'-BNA^{NC} backbone modification of TFO could be a key chemical modification and may eventually lead to progress in therapeutic applications of the antigene strategy *in vivo*.

INTRODUCTION

In recent years, triplex DNA has attracted considerable interest because of its possible biological function *in vivo* and its wide variety of potential applications, such as regulation of gene expression, site-specific cleavage of DNA, and mapping of genomic DNA. A triplex is usually formed through the sequence-specific interaction of a single-stranded homopurine or homopyrimidine triplex-forming oligonucleotide (TFO) with the major groove of homopurine-homopyrimidine stretch in duplex DNA.[1] In the pyrimidine motif triplex, a homopyrimidine TFO binds parallel to the homopurine strand of the target duplex by Hoogsteen hydrogen bonding to form T•A:T and C^+•G:C triplets.[1] As protonation of the cytosine bases in TFO is required to bind with the guanine bases of the G:C duplex, the pyrimidine motif triplex is usually formed at acidic pH and unstable at neutral pH.[1] However, stabilization of the pyrimidine motif triplex at neutral pH is quite necessary for its applicability as an antigene drug *in vivo*. We have previously shown that 2'-*O*,4'-*C*-methylene bridged nucleic acid (2',4'-BNA) backbone modification of TFO (Fig. 1) increased the thermal stability of the pyrimidine motif triplex and the binding constant of the pyrimidine motif triplex formation at neutral pH.[2,3] Previous studies have shown that another backbone modification using 2'-*O*,4'-*C*-aminomethylene bridged nucleic acid (2',4'-BNA^{NC}) (Fig. 1) has been developed.[4-6] Here, using a 23-bp target duplex and a 15-mer homopyrimidine TFO, we have examined the thermodynamic effect of the 2',4'-BNA^{NC} backbone modification of TFO on the pyrimidine motif triplex formation at neutral pH. The thermodynamic properties were analyzed by UV melting. We have found a significant effect of the 2',4'-BNA^{NC} backbone modification of TFO on the promotion of the pyrimidine motif triplex formation at neutral pH.

MATERIALS AND METHODS

We synthesized a 15-mer homopyrimidine TFO, Pyr15TM: 5'-^{m}CT^{m}CTT^{m}CTTTT^{m}CTTT^{m}C-3' (^{m}C=5-methylcytidine), and 23-mer complementary DNA oligonucleotides for the target duplex, Pur23A: 5'-GCGCGAGAAGAAAAGAAAGCCGG-3' and Pyr23T: 5'-CCGGCTTTCTTTTCTTCTCGCGC-3', on a DNA synthesizer and purified them with a reverse-phase HPLC. Pur23A and Pyr23T were annealed by heating at up to 90 °C, followed by a gradual cooling to room temperature. The annealed sample was applied on a hydroxyapatite column to remove unpaired single strands. The 15-mer 2',4'-BNA modified homopyrimidine TFOs, Pyr15BNA7-1: 5'-$\underline{^{m}C}$T$\underline{^{m}C}$T$\underline{T}^{m}$C$\underline{T}$T$\underline{T}$T$\underline{^{m}C}$T$\underline{T}$T^{m}C-3', Pyr15BNA7-2: 5'-^{m}C$\underline{T}^{m}$C$\underline{T}$T$\underline{^{m}C}$T$\underline{T}$T$\underline{T}^{m}$C$\underline{T}$T$\underline{^{m}C}$-3', Pyr15BNA5-1: 5'-$\underline{^{m}C}$T^{m}C$\underline{T}^{m}$C$\underline{T}$T$\underline{T}^{m}$C$\underline{T}$T$\underline{T}^{m}$C$\underline{T}$T^{m}C-3', Pyr15BNA5-2: 5'-^{m}C$\underline{T}^{m}$C$\underline{T}^{m}$C$\underline{T}$T$\underline{T}$T$\underline{^{m}C}$TT$\underline{T}^{m}$C-3', and Pyr15BNA5-3: 5'-^{m}CT^{m}CTT$\underline{^{m}C}$$\underline{TTTT}$^{m}CTTT^{m}C-3' (^{m}C=5-methylcytidine, $\underline{T}$=2',4'-BNA modified T,

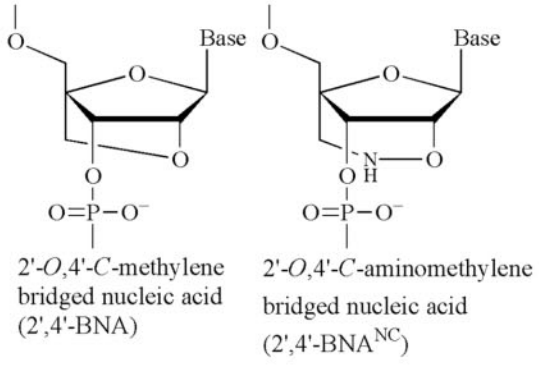

Fig. 1. Structural formulas of oligonucleotides.

Downloaded from http://nass.oxfordjournals.org/ by guest on March 25, 2013

Table 1: Melting temperatures of the triplex involving each of the unmodified (Pyr15TM), 2',4'-BNA modified (Pyr15BNA7-1, Pyr15BNA7-2, Pyr15BNA5-1, Pyr15BNA5-2, Pyr15BNA5-3), or 2',4'-BNA^{NC} modified (Pyr15BNANC7-1, Pyr15BNANC7-2, Pyr15BNANC5-1, Pyr15BNANC5-2, Pyr15BNANC5-3) TFO at pH 6.8[a], obtained from UV melting.

TFO	T_{m1} (°C)	T_{m2} (°C)	TFO	T_{m1} (°C)	T_{m2} (°C)
Pyr15TM	37.5	74.0			
Pyr15BNA7-1	72.0[b]		Pyr15BNANC7-1	77.7[b]	
Pyr15BNA7-2	73.0[b]		Pyr15BNANC7-2	83.4[b]	
Pyr15BNA5-1	73.3[b]		Pyr15BNANC5-1	74.3[b]	
Pyr15BNA5-2	72.0[b]		Pyr15BNANC5-2	72.7[b]	
Pyr15BNA5-3	52.3	74.2	Pyr15BNANC5-3	73.6[b]	

[a]10 mM sodium cacodylate-cacodylic acid, 200 mM sodium chloride, and 20 mM magnesium chloride (pH 6.8). [b]One step transition corresponds to a direct melting of the triplex to its constituting single strand oligonucleotides.

$^{m}\underline{\underline{C}}$=2',4'-BNA modified 5-methylcytidine), were prepared as described previously.[2,3] The 15-mer 2',4'-BNA^{NC} modified homopyrimidine TFOs, Pyr15BNANC7-1: 5'-$^{m}\underline{\underline{C}}T^{m}\underline{\underline{C}}$TT^{m}C$\underline{\underline{T}}TT^{m}\underline{\underline{C}}$TT^{m}C-3', Pyr15BNANC7-2: 5'-^{m}C$\underline{\underline{T}}^{m}C\underline{\underline{T}}T^{m}\underline{\underline{C}}T\underline{\underline{T}}T\underline{\underline{T}}^{m}C\underline{\underline{T}}T\underline{\underline{T}}^{m}$C-3', Pyr15BNANC5-1: 5'-$^{m}\underline{\underline{C}}$T^{m}C$\underline{\underline{T}}$T^{m}C$\underline{\underline{T}}TT\underline{\underline{T}}^{m}CT\underline{\underline{T}}$T^{m}C-3', Pyr15BNANC5-2: 5'-^{m}C$\underline{\underline{T}}^{m}CT\underline{\underline{T}}^{m}CT\underline{\underline{T}}TT^{m}\underline{\underline{C}}TT\underline{\underline{T}}^{m}$C-3', and Pyr15BNANC5-3: 5'-^{m}CT^{m}CTT$^{m}\underline{\underline{C}}$TTTT^{m}CTTT^{m}C-3' (^{m}C=5-methylcytidine, $\underline{\underline{T}}$=2',4'-BNA^{NC} modified T, $^{m}\underline{\underline{C}}$=2',4'-BNA^{NC} modified 5-methylcytidine) were prepared as described previously.[4-6] UV melting experiments were performed on a DU-640 spectrophotometer (Beckman Inc.) equipped with a Peltier type cell holder.

RESULTS AND DISCUSSION

The thermal stability of the triplex involving each of the unmodified (Pyr15TM), 2',4'-BNA modified (Pyr15BNA 7-1, Pyr15BNA7-2, Pyr15BNA5-1, Pyr15BNA5-2, Pyr15 BNA5-3), or 2',4'-BNA^{NC} modified (Pyr15BNANC7-1, Pyr15BNANC7-2, Pyr15BNANC5-1, Pyr15BNANC5-2, Pyr15BNANC5-3) TFOs was investigated at pH 6.8 by UV melting (Table 1). The triplex involving Pyr15TM showed two-transition melting profile. The first transition at lower temperature, T_{m1}, (37.5 °C) was the dissociation of the triplex to the target duplex and the TFO, and the second transition at higher temperature, T_{m2}, (74.0 °C) was the melting of the target duplex. On the other hand, the triplex involving each of the 2',4'-BNA modified TFOs (Pyr15 BNA7-1, Pyr15BNA7-2, Pyr15BNA5-1, Pyr15BNA5-2) showed only one transition at the higher temperature, T_m. As the magnitude in UV absorbance change at T_m was almost equal to the sum of those at T_{m1} and T_{m2}, the transition was identified as a direct melting of the triplex to its constituting single strands. Thus, the 2',4'-BNA modification of TFO (Pyr15BNA7-1, Pyr15BNA7-2, Pyr15BNA5-1, Pyr15BNA5-2) increased T_{m1} by about 35 °C. Although the triplex involving Pyr15BNA5-3 showed two-transition melting profile unlike the case of the other 2',4'-BNA modified TFOs, T_{m1} was increased by about 15 °C. The increase in T_{m1} by the 2',4'-BNA modification of TFO was quite consistent with the previous results.[3]

Similar to the case of the 2',4'-BNA modified TFOs, the triplex involving each of the 2',4'-BNA^{NC} modified TFOs (Pyr15BNANC7-1, Pyr15BNANC7-2, Pyr15BNANC5-1, Pyr15BNANC5-2, Pyr15BNANC5-3) showed only one transition at the higher temperature, which corresponds to a direct melting of the triplex to its constituting single strands. The 2',4'-BNA^{NC} modification of TFO increased T_{m1} by more than 35 °C. The present results indicate that the 2',4'-BNA^{NC} modification of TFO increased the thermal stability of the pyrimidine motif triplex at neutral pH.

CONCLUSION

We conclude that the 2',4'-BNA^{NC} backbone modification of TFO could be a key chemical modification and may eventually lead to progress in therapeutic applications of the antigene strategy *in vivo*.

REFERENCES

1. Soyfer, V. N., Potaman, V. N. (1996) *Triple-Helical Nucleic Acids*, Springer-Verlag New York, Inc.
2. Obika, S., Nanbu, D., Hari, Y., Morio, K., In, Y., Ishida, T., Imanishi, T. (1997) *Tetrahedron Lett.* **38,** 8735-8738.
3. Torigoe, H., Hari, Y., Sekiguchi, M., Obika, S., Imanishi, T. (2001) *J. Biol. Chem.* **276,** 2354-2360.
4. Rahman, S. M. A., Seki, S., Obika, S., Haitani, S., Miyashita, K., Imanishi, T. (2007) *Angew. Chem. Int. Ed.*, **46**, 4306-4309.
5. Miyashita, K., Rahman, S. M. A., Seki, S., Obika, S., Imanishi, T. (2007) *Chem. Commun.* 3765-3767.
6. Rahman, S. M. A., Seki, S., Obika, S., Yoshikawa, H., Miyashita, K., Imanishi, T. (2008) *J. Am. Chem. Soc.*, **130**, 4886-4896.

*Corresponding author. E-mail: htorigoe@rs.kagu.tus.ac.jp

Downloaded from http://nass.oxfordjournals.org/ by guest on March 25, 2013