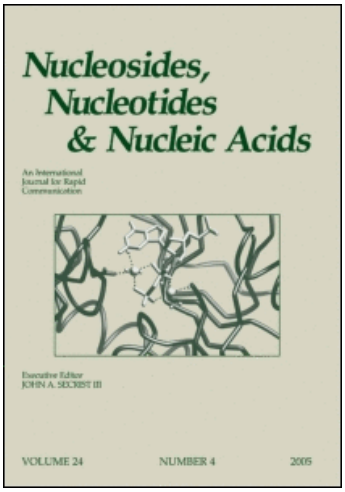

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Triplex Formation Involving 2′-*O*,4′-*C*-Methylene Bridged Nucleic Acid (2′,4′-BNA) with 1-Isoquinolone Base Analogue: Efficient and Selective Recognition of C:G Interruption

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ABSTRACT

For the effective recognition of C•G interruption in homopurine-homopyrimidine duplex DNA, we examined triplex-forming ability and sequence-selectivity of a triplex-forming oligonucleotide (TFO) involving of 2′-*O*, 4′-*C*-methylene bridged nucleic acid with 1-isoquinolone base analogue. We found that the modified TFO formed stable triplex with high binding affinity and sequence-selectivity.

Key Words: Triplex; 2′-*O*, 4′-*C*-Methylene bridged nucleic acid; 1-Isoquinolone.

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INTRODUCTION

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. A triplex is 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. One major limitation of triplex formation is that only purine bases in the homopurine strand of the target duplex are usually possible to be recognized by TFO.[1] Recognition of pyrimidine bases is hard to achieve and restricts triplex formation to homopurine-homopyrimidine target sites.[1] Overcoming this restriction to include recognition of pyrimidine bases is quite necessary for the applicability of the triplex as an antigene drug in vivo.

RESULTS AND DISCUSSION

We examined the thermodynamic properties of 2′-*O*,4′-*C*-methylene bridged nucleic acid (2′,4′-BNA) containing 1-isoquinolone as a nucleobase (Q^B) to recognize a C interruption in the homopurine strand of the target duplex for pyrimidine motif triplex formation at neutral pH (Fig. 1).[2–5] Table 1 summarizes the binding constant for the triplex formation between a 15-mer TFO, Pyr15X:5′-TTTTT*C*TXT-*C*T*C*T*C*T-3′ [*C* = 5-methylcytidine, X = T, H^B(2′,4′-BNA containing abasic site), or Q^B], and a 21-bp target duplex, Pur21Y/Pyr21Z:5′-GCTAAAAAGAYAGAGA-GATCG-3′/3′-CGATTTTTCTZTCTCTCTAGC-5′ [Y:Z = C:G, G:C, T:A or A:T] at 25°C and pH6.8, obtained from isothermal titration calorimetry.[6] The binding constant of the triplex formation involving $X \bullet Y : Z = Q^B \bullet C : G$ triad was at least 2.2-times larger than those involving $X \bullet Y : Z = Q^B \bullet G : C$, $Q^B \bullet T : A$, or $Q^B \bullet A : T$ triad. Thus, the triplex formation involving TFO with Q^B is highly sequence-

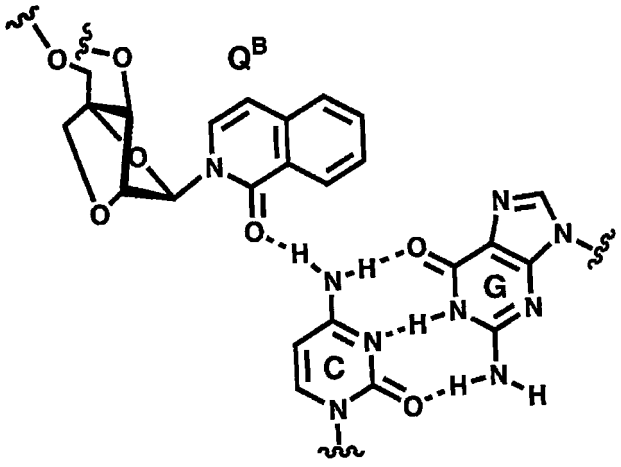

Figure 1. Proposed recognition scheme for $Q^B C : G$ base triplet.

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Table 1. Binding constant (M^{-1}) for the triplex formation between a 15-mer TFO (X = T, H^B, or Q^B) and a 21-bp target duplex (Y : Z = C : G, G : C, T : A, or A : T) at 25°C and pH 6.8[a], obtained from ITC.

	Y:Z			
X	C:G	G:C	T:A	A:T
T	3.23×10^7	1.15×10^7	8.73×10^6	9.05×10^7
H^B	2.19×10^7	1.68×10^7	1.77×10^7	3.53×10^6
Q^B	6.26×10^7	2.81×10^7	1.23×10^7	1.22×10^7

[a]7 mM sodium cacodylate-cacodylic acid, 140 mM potassium chloride and 10 mM spermine (pH 6.8).

selective to specifically recognize C:G target base pair. In addition, X • Y : Z = Q^B • C : G triad gave 1.9-times larger binding constant than X • Y : Z = T • C : G triad, which has been known to be the most stable combination in natural base • C : G triad.[7] Our results certainly support the idea that Q^B could be a key nucleoside to recognize a C interruption in the homopurine strand of the target duplex with high binding affinity and selectivity and reduce the restriction of target sequences for triplex formation.

REFERENCES

1. Gowers, D.M.; Fox, K.R. Towards mixed sequence recognition by triple helix formation. Nucleic Acids Res. **1999**, *27*, 1569–1577.
2. Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. Synthesis of 2′-*O*,4′-*C*-methyleneuridine and -cytidine: novel bicyclic nucleosides having a fixed C_3′-*endo* sugar puckering. Tetrahedron Lett. **1997**, *38*, 8735–8738.
3. Obika, S.; Hari, Y.; Sekiguchi, M.; Imanishi, T. A 2′,4′-Bridged nucleic acid containing 2-pyridone as a nucleobase: efficient recognition of a C • G interruption by triplex formation with a pyrimidine motif. Angew. Chem., Int. Ed. Engl. **2001**, *40*, 2079–2081.
4. Torigoe, H.; Hari, Y.; Sekiguchi, M.; Obika, S.; Imanishi, T. 2′-*O*,4′-*C*-Methylene bridged nucleic acid modification promotes pyrimidine motif triplex DNA formation at physiological pH. J. Biol. Chem. **2001**, *276*, 2354–2360.
5. Torigoe, H.; Hari, Y.; Obika, S.; Imanishi, T. Triplex formation involving 2′,4′-BNA with 2-pyridone base analogue: efficient and selective recognition of C:G interruption. Nucleic Acids Res. **2001**, *Sup. 1*, 281–282.
6. Kamiya, M.; Torigoe, H.; Shindo, H.; Sarai, A. Temperature dependence and sequence specificity of DNA triplex formation: an analysis using isothermal titration calorimetry. J. Am. Chem. Soc. **1996**, *118*, 4532–4538.
7. Yoon, K.; Hobbs, C.A.; Koch, J.; Sardaro, M.; Kutny, R.; Weis, A.L. Elucidation of the sequence-specific third-strand recognition of four Watson-Crick base pairs in a pyrimidine triple-helix motif: T • AT, C • GC, T • CG, and G • TA. Proc. Natl. Acad. Sci. USA **1992**, *89*, 3840–3844.

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