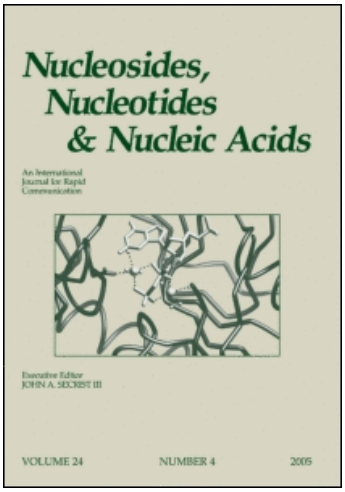

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PROMOTION OF TRIPLEX FORMATION BY 2′-*O*,4′-*C*-METHYLENE BRIDGED NUCLEIC ACID (2′,4′-BNA) MODIFICATION: THERMODYNAMIC AND KINETIC STUDIES

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ABSTRACT

We analyzed the effect of 2′-*O*,4′-*C*-methylene bridged nucleic acid (2′,4′-BNA) modification of triplex-forming oligonucleotide (TFO) on pyrimidine motif triplex formation at neutral pH, a condition where pyrimidine motif triplexes are unstable. The binding constant of the pyrimidine motif triplex formation at pH 6.8 with 2′,4′-BNA modified TFO was about 20 times larger than that observed with unmodified TFO. The observed increase in the binding constant at neutral pH by the 2′,4′-BNA modification resulted from the considerable decrease in the dissociation rate constant.

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 (1–3). A triplex is usually formed through the sequence-specific interaction of a single-stranded homopurine or homopyrimidine triplex-forming

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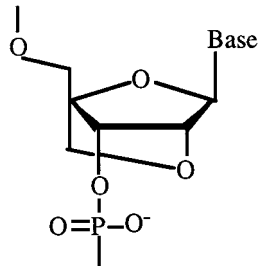

Figure 1. Nucleotide unit of 2′-*O*,4′-*C*-methylene bridged nucleic acid (2′,4′-BNA).

oligonucleotide (TFO) with the major groove of homopurine-homopyrimidine stretch in duplex DNA (1–3). 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 (1–3). As protonation of the cytosine base in TFO is required to bind with the guanine base of the G:C duplex, the pyrimidine motif triplex is usually formed at acidic pH and unstable at neutral pH (4–6). 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) modification of TFO (Fig. 1) increased the thermal stability of the pyrimidine motif triplex between a homopurine-homopyrimidine target duplex and its specific cytosine-rich TFO at neutral pH (7,8). Here, using a 23-bp target duplex and a 15-mer homopyrimidine TFO, we have further extended our study to explore the thermodynamic and kinetic effects of the 2′,4′-BNA modification of TFO on the pyrimidine motif triplex formation at pH 6.8. The thermodynamic and kinetic properties have been analyzed by isothermal titration calorimetry (ITC) and interaction analysis system (IAsys), respectively (9,10). We have found a significant effect of the 2′,4′-BNA modification of TFO on the promotion of the pyrimidine motif triplex formation at neutral pH.

MATERIALS AND METHODS

We synthesized a 15-mer TFO, Pyr15T: 5′-CTCTTCTTTTCTTTC-3′, and complementary 23-mer DNA oligonucleotides, Pur23A: 5′-GCGCGAGAAGAA-AAGA AAGCCGG-3′ and Pyr23T: 5′-CCGGCTTTCTTTTCTTCTCGCGC-3′, on a DNA synthesizer and purified them with a reverse-phase HPLC. The 2′,4′-BNA modified TFOs, Pyr15BNA7-1: 5′-CTCTTCT TTTCTTTC-3′, Pyr15BNA 7-2: 5′-CTCTTCTTTT CTTTC-3′, Pyr15BNA5-1: 5′-C TCTTCTTTTCTTTC-3′ and Pyr15BNA5-2: 5′-CTCTTCTTTTCTTT C-3′ (modified positions are underlined), were synthesized as described previously (7). Thermodynamic experiments were carried out on a MCS ITC system (Microcal Inc., U. S. A.) (9,10). Kinetic analyses were performed on an IAsys instrument (Affinity Sensors Cambridge, U. K.), where a real time biomolecular interaction was measured with a laser biosensor (10).

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RESULTS AND DISCUSSION

Table 1 summarizes the thermodynamic parameters of the triplex formation between TFO (Pyr15T, Pyr15BNA7-1, Pyr15BNA7-2, Pyr15BNA5-1 or Pyr15BNA5-2) and a target duplex (Pur23A•Pyr23T) at 25°C, obtained from ITC. The signs of both ΔH and ΔS were negative under all the conditions. Since an observed negative ΔS was unfavorable for the triplex formation, the triplex formation was driven by a large negative ΔH. The K_a for Pyr15T at pH 5.8 was about 20 times larger than that observed at pH 6.8, which was consistent the previously reported results (4–6,10) showing that neutral pH is unfavorable for pyrimidine motif triplex formation involving C^+•GC triads. In addition, the K_a for Pyr15BNA7-1, Pyr15BNA7-2, Pyr15BNA5-1 or Pyr15BNA5-2 at pH 6.8 was 10–20 times larger than that observed for Pyr15T at pH 6.8. Thus, the 2′,4′-BNA modification of TFO increased the K_a for the pyrimidine motif triplex formation at neutral pH by 10–20 times. The increase in the K_a by the 2′,4′-BNA modification was similar in magnitude among the four modified TFOs, suggesting that the number and position of the modification did not significantly affect the magnitudes of the increase in K_a. Although the K_a and ΔG were similar between the four modified TFOs at pH 6.8 and Pyr15T at pH 5.8, the ingredients of ΔG, that is, ΔH and ΔS, were obviously different from each other. The magnitudes of the negative ΔH and ΔS for the four modified TFOs at pH 6.8 were smaller than those observed for Pyr15T at pH 5.8, indicating that the triplex formation with the 2′,4′-BNA modified TFO was entropically more favorable than that with the unmodified TFO. The more rigidity of the 2′,4′-BNA modified TFO in the free state may result in the smaller entropic loss upon the triplex formation.

Table 2 summarizes the kinetic parameters of the same triplex formation at 25°C and pH 6.8, obtained from IAsys. The magnitudes of K_a calculated from k_{assoc}/k_{dissoc} in Table 2 were consistent with those in Table 1. Although the k_{assoc}

Table 1. Thermodynamic Parameters for the Triplex Formation Between a 15-mer TFO (Pyr15T, Pyr15BNA7-1, Pyr15BNA7-2, Pyr15BNA5-1 or Pyr15BNA5-2) and a 23-bp Target Duplex (Pur23A•Pyr23T) at 25 °C, Obtained from ITC

TFO	pH	K_a (M^{-1})	K_a (Relative)	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})
Pyr15T	5.8[a]	3.83×10^6	19.4	−8.98	−85.6	−257
Pyr15T	6.8[b]	1.97×10^5	1.0	−7.22	−34.9	−92.7
Pyr15BNA7-1	6.8[b]	2.28×10^6	11.6	−8.67	−55.5	−157
Pyr15BNA7-2	6.8[b]	2.16×10^6	11.0	−8.64	−57.3	−163
Pyr15BNA5-1	6.8[b]	3.65×10^6	18.5	−8.95	−60.7	−174
Pyr15BNA5-2	6.8[b]	2.06×10^6	10.5	−8.61	−60.5	−174

[a] 10 mM sodium cacodylate-cacodylic acid, 200 mM sodium chloride and 20 mM magnesium chloride (pH 5.8).
[b] 10 mM sodium cacodylate-cacodylic acid, 200 mM sodium chloride and 20 mM magnesium chloride (pH 6.8).

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Table 2. Kinetic Parameters for the Triplex Formation Between a 15-mer TFO (Pyr15T, Pyr15BNA-7-1, Pyr15BNA7-2, Pyr15BNA5-1 or Pyr15BNA5-2) and a 23-bp Target Duplex (Pur23A•Pyr23T) at 25°C and pH 6.8[a], Obtained from IAsys

TFO	k_{assoc} ($M^{-1}s^{-1}$)	k_{assoc} (Relative)	k_{dissoc} (s^{-1})	k_{dissoc} (Relative)	K_a (M^{-1})	K_a (Relative)
Pyr15T	6.31×10^2	1.0	1.17×10^{-2}	1.0	5.41×10^4	1.0
Pyr15BNA7-1	4.86×10^2	0.77	1.57×10^{-4}	0.013	3.09×10^6	57.1
Pyr15BNA7-2	5.05×10^2	0.80	2.35×10^{-4}	0.020	2.15×10^6	39.7
Pyr15BNA5-1	5.93×10^2	0.94	2.97×10^{-4}	0.025	2.00×10^6	37.0
Pyr15BNA5-2	6.32×10^2	1.0	2.66×10^{-4}	0.023	2.38×10^6	44.0

[a] 10 mM sodium cacodylate-cacodylic acid, 200 mM sodium chloride and 20 mM magnesium chloride (pH 6.8).

for the four modified TFOs were smaller than that for Pyr15T, the k_{dissoc} were significantly decreased by the 2′,4′-BNA modification of TFO. Thus, the larger K_a by the 2′,4′-BNA modification resulted from the decrease in k_{dissoc}. Maintaining the triplex structure is the effect of the 2′,4′-BNA modification to increase in K_a. The present results indicate that the 2′,4′-BNA modification of TFO can increase the binding constant of the pyrimidine motif triplex formation at pH 6.8 by about 20 times with the decrease in k_{dissoc}, which may lead to progress in therapeutic applications of the antigene strategy *in vivo*.

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