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Triplex formation involving 2',4'-BNA with 2-pyridone base analogue: Efficient and selective recognition of C:G interruption

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ABSTRACT

We examined the thermodynamic properties of 2',4'-bridged nucleic acid containing 2-pyridone as a nucleobase (P^B) to recognize a C interruption in the homopurine strand of the target duplex for pyrimidine motif triplex formation at neutral pH. The triplex formation involving triplex-forming oligonucleotide with P^B is highly sequence-selective to specifically recognize C:G target base pair rather than the other G:C, T:A, or A:T base pairs. P^B•C:G triad gives significantly larger binding constant than T•C:G triad, which has been known to be the most stable combination in natural base•C:G triad. Our results certainly support the idea that P^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.

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). 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. 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). 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, 2). Recognition of pyrimidine bases is hard to achieve and usually restricts triplex formation to homopurine-homopyrimidine target sites (1, 2). Overcoming this restriction to include recognition of pyrimidine bases is quite necessary for the applicability of the triplex as an antigene drug *in vivo*. We have previously shown that 2',4'-bridged nucleic acid (2',4'-BNA) containing 2-pyridone as a nucleobase (P^B) (Fig. 1) efficiently recognizes a C interruption in the homopurine strand of the target duplex for pyrimidine motif triplex formation at neutral pH (3-6). Here, we have further extended our study to explore the thermodynamic effects of P^B on pyrimidine motif triplex formation at pH 6.8 (Fig. 2). The thermodynamic properties have been analyzed by isothermal titration calorimetry (ITC) (6, 7). We have found that P^B recognizes a C interruption in the homopurine strand of the target duplex with high binding affinity and selectivity.

MATERIALS AND METHODS

We synthesized a 15-mer TFO, Pyr15X: 5'-TTTTTCTXTC TCTCT-3' (C=5-methylcytidine, **X**=T), and complementary 21-mer DNA oligonucleotides, Pur21Y: 5'-GCTAAAAAGA **Y**AGAGAGATCG-3' (**Y**=A, C, G, T) and Pyr21Z: 5'-CGA TCTCTCT**Z**TCTTTTTAGC-3' (**Z**=A, C, G, T), on a DNA synthesizer and purified them with a reverse-phase HPLC. Modified TFOs, where **X**=2',4'-BNA containing abasic site (H^B), **X**=DNA containing 2-pyridone (P), or **X**=P^B, were synthesized as described previously (3-5). Thermodynamic experiments were carried out on a MCS ITC system (Microcal Inc., U. S. A.) (6, 7).

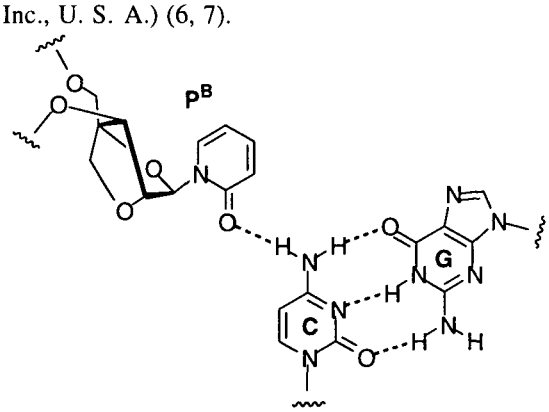

Fig. 1: Proposed recognition scheme for P^B•C:G base triplet

Table 1: Binding constant for the triplex formation between a 15-mer TFO (**X**=T, H^B, P^B, or P) 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 X 10^7	1.15 X 10^7	8.73 X 10^6	9.05 X 10^7
H^B	2.19 X 10^7	1.68 X 10^7	1.77 X 10^7	3.53 X 10^6
P^B	6.30 X 10^7	1.73 X 10^7	1.25 X 10^7	9.15 X 10^6
P	1.48 X 10^7	6.30 X 10^6	6.61 X 10^6	7.45 X 10^6

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

Table 2: Thermodynamic parameters for the triplex formation between a 15-mer TFO (**X**=T, H^B, P^B, or P) and a 21-bp target duplex (Pur21C:Pyr21G) at 25 °C and pH 6.8[a], obtained from ITC

X	K_a (M^{-1})	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})
T	3.23 x 10^7	-10.2	-69.9	-200
H^B	2.19 x 10^7	-10.0	-88.6	-264
P^B	6.30 x 10^7	-10.6	-97.4	-291
P	1.48 x 10^7	- 9.8	-122	-375

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

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Pyr15X:     5'-TTTTTC̲TXTC̲TC̲TC̲T-3'
Pur21Y: 5'-GCTAAAAAGAYAGAGAGATCG-3'
Pyr21Z: 3'-CGATTTTTCTZTCTCTCTAGC-5'
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Fig. 2: Oligonucleotide sequences of the target duplex (**Y**:**Z**=C:G, G:C, T:A, or A:T) and its specific TFO (C̲=5-methylcytidine, **X**=T, H^B, P^B, or P)

RESULTS AND DISCUSSION

Table 1 summarizes the binding constant for the triplex formation between a 15-mer TFO (**X**=T, H^B, P^B, or P) and a 21-bp target duplex (**Y**:**Z**=C:G, G:C, T:A, or A:T) at 25 °C and pH 6.8 (Fig. 2), obtained from ITC. When **X**=T, **Y**:**Z**=A:T shows the largest binding constant for the triplex formation, which is consistent with the previous result that T•A:T and C^+•G:C are the most stable base triplets for the pyrimidine motif triplex formation (1, 2). On the other hand, when **X**=P^B and **X**=P, the binding constant for **Y**:**Z**=C:G is significantly larger than those for the other target base pairs, indicating that the triplex formation involving TFO with **X**=P^B and **X**=P is highly sequence-selective to specifically recognize C:G target base pair. Furthermore, P^B•C:G triad gives significantly larger binding constant than T•C:G and P•C:G triads, although T•C:G has been known to be the most stable combination in natural base•C:G triad (8). These results clearly indicate that P^B recognizes C:G target base pair for the pyrimidine motif triplex formation with high binding affinity and selectivity.

To understand the mechanism of the recognition by P^B, Table 2 summarizes the thermodynamic parameters for the triplex formation involving TFO (**X**=T, H^B, P^B, or P) to recognize C:G target base pair. The magnitude of the negative ΔH for P^B•C:G is larger than that for H^B•C:G by 9 kcal mol^{-1}. The hydrogen bonding and the base stacking involved in the triplex formation are usually considered to be the major sources of the negative ΔH upon the triplex formation (7). Thus, the difference in ΔH may correspond to the hydrogen bonding and the base stacking of P nucleobase upon the triplex formation. In addition, the magnitude of the negative ΔS for P^B•C:G is smaller than that for P•C:G. The negative ΔS upon the triplex formation is mainly contributed by a negative conformational entropy change due to the conformational restraint of TFO involved in the triplex formation (7). We have previously reported that the BNA-modified TFO in the free state may be more rigid than the corresponding unmodified TFO (6). The more rigidity of the BNA-modified TFO in the free state causes the smaller entropic loss upon the triplex formation. The present results suggest that P^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.

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