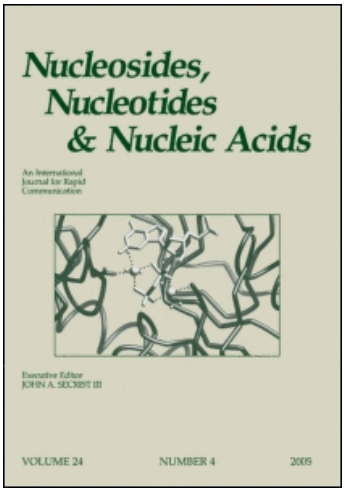

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Torben Højland[a]; B. Ravindra Babu[a]; Torsten Bryld[a]; Jesper Wengel[a]
[a] Nucleic Acid Center, University of Southern Denmark, Odense M, Denmark

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TRIPLEX-FORMING ABILITY OF MODIFIED OLIGONUCLEOTIDES

Torben Højland, B. Ravindra Babu, Torsten Bryld, and Jesper Wengel □
Nucleic Acid Center, University of Southern Denmark, Odense M, Denmark

□ *We present our studies on the ability of several different nucleotide analogs as triplex-forming oligonucleotides. The modifications tested include 4′-C-hydroxymethyl, LNA, 2′-amino-LNA and N2′-functionalized 2′-amino-LNA. Triplexes containing monomers of N2′-glycyl-functionalized 2′-amino-LNA are particularly stable.*

Keywords TFO; triplex; conformationally restricted nucleosides

INTRODUCTION

A triplex-forming oligonucleotide (TFO) is an oligonucleotide that can bind to double stranded DNA and form a triplex. In recent years modifications in the sugar units of TFOs have resulted in triplexes that are much more stable than their unmodified counterparts.[1] Here, we report the hybridization studies of some triplexes containing TFOs with modified sugar units. The target duplex (Figure 1) contains the polypurine tract target sequence of HIV-1.[2,3]

RESULTS AND DISCUSSION

TFOs containing the 4′-*C*-hydroxymethyl modification form triplexes that are slightly more stable than the unmodified reference triplex (Table 1). The introduction of LNA in TFOs leads to a high stabilization of the triplex compared to the DNA reference. This is in good agreement with what has previously been reported.[4–6] The incorporation of the amino analog of LNA, 2′-amino-LNA,[7] also results in triplexes melting at relatively high temperatures. However, 2′-amino-LNA is not as stabilizing to the triplex as LNA itself.

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Address correspondence to Torben Højland, Nucleic Acid Center, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark. E-mail: tohoe00@student.sdu.dk

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3′- GGT  GAA  AAA  TTT  TCT  TTT  CCC  CCC  TGA  CC
5′- CCA  CTT  TTT  AAA  AGA  AAA  GGG  GGG  ACT  GG
                  . . .  . . .  . . .  . . .  . . .  .
                5′- TTT  TCT  TTT  CCC  CCC  T
```

FIGURE 1 Sequence of unmodified triplex.

TABLE 1 Melting temperature of triplexes

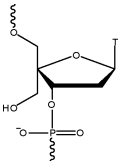
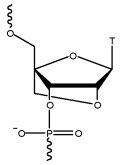
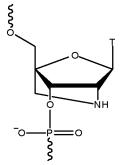
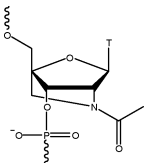
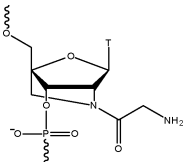
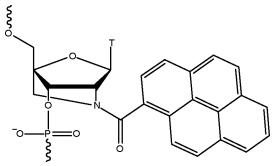
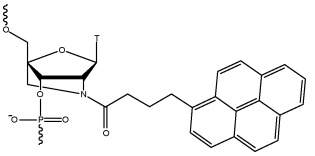
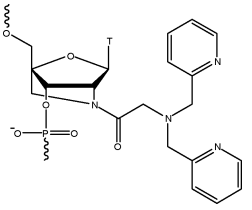
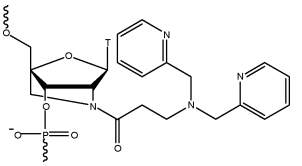
Modification	Sequence	pH 7.0		pH 6.0	
		T_m	ΔT_m/mod	T_m	ΔT_m/mod
None	TTT TCT TTT CCC CCC T	n.t.	—	27.0	—
	TTT TC**X** TTT CCC CCC T	n.t.	—	28.0	1.0
	TT**X** TC**X** TT**X** CCC CCC T	n.t.	—	28.0	0.3
	TTT TCT **XXX** CCC CCC T	n.t.	—	28.5	0.5
	TTT TCT TT**X** CCC CCC T	9.5	>4.5	37.0	10.0
	TT**X** TC**X** TT**X** CCC CCC T	23.0	>6.0	48.0	7.0
	TTT TCT TT**X** CCC CCC T	n.t.	—	36.0	9.0
	TT**X** TC**X** TT**X** CCC CCC T	16.0	>3.7	46.0	6.3
	TTT TCT TT**X** CCC CCC T	11.5	>6.5	37.0	10.0
	TT**X** TC**X** TT**X** CCC CCC T	24.0	>6.3	46.0	6.3
	TTT TCT TT**X** CCC CCC T	13.0	>8.0	38.5	11.5
	TT**X** TC**X** TT**X** CCC CCC T	28.0	>7.7	52.0	8.3
	TTT TCT TT**X** CCC CCC T	n.t.	—	29.5	2.5
	TT**X** TC**X** TT**X** CCC CCC T	n.t.	—	n.t.	<−7.3

TABLE 1 (*Continued*)

Modification	Sequence	pH 7.0 T_m	pH 7.0 ΔT_m/mod	pH 6.0 T_m	pH 6.0 ΔT_m/mod
	TTT TCT TT**X** CCC CCC T	n.t.	—	25.5	−1.5
	TT**X** TC**X** TT**X** CCC CCC T	n.t.	—	n.t.	<−7.3
	TTT TCT TT**X** CCC CCC T	n.t.	—	41.0	14.0
	TTT TCT TT**X** CCC CCC T	n.t.	—	38.0	11.0

T_m values were obtained in a buffer containing 10 mM cacodylate, 150 mM NaCl and 10 mM $MgCl_2$. The concentration of the duplex was 1 μM and the concentration of the TFO was 1.5 μM. n.t. = no transition observed above 5°C.

N2′-functionalization of the 2′-amino-LNA monomer with pyrenes induces a destabilization of the triplex compared to the corresponding unfunctionalized 2′-amino-LNA triplexes. At pH 6.0, with TFOs containing one pyrene-functionalized 2′-amino-LNA monomer, the triplex is as thermally stable as the unmodified triplex. However, when three of these pyrene monomers are incorporated into the TFO no triplex-to-duplex transition above 5°C can be detected.

However, when 2′-amino-LNA monomers functionalized with acetyl groups are introduced in the TFO the resulting triplex is further stabilized, and the stabilization is roughly the same as for LNA.

Even further stabilization of the triplex is seen when 2′-amino-LNA is N2′-functionalized with a glycyl group. It is likely that this stabilization is the result of protonation of the distal amino group. It has previously been shown that a TFO containing four incorporations of a 2′-aminoethoxy T-monomer forms a quite stable triplex compared to the unmodified triplex.[8] One could imagine that the very high stability of triplexes produced with the glycyl-functionalized 2′-amino-LNA monomer is a consequence of combining both favorable conformational restriction and an electrostatic interaction between the protonated amine and the negatively charged backbone.

Similar factors could be in play for 2′-amino-LNA functionalized with *N,N*-bis(2-pyridylmethyl)glycyl. Interestingly, no melting temperature is observed above 5°C at pH 7.0, but at pH 6.0 the highest ΔT_m/modification of the whole study is observed. 2′-amino-LNA functionalized with *N,N*-bis(2-pyridylmethyl)-β-alanyl shows the same trend, although the ΔT_m/modification is not as prominent as for *N,N*-bis(2-pyridylmethyl)glycyl. This is likely because the distance between the 2′-amino-LNA skeleton and the bis(2-pyridylmethyl) unit is more optimal with the glycyl linker.

CONCLUSION

We have studied the triplex-forming ability of some modifications and found that very stable triplexes are formed when LNA and 2′-amino-LNA monomers are introduced into the TFO. In particular, TFOs containing monomers of 2′-amino-LNA N2′-functionalised with glycyl lead to highly stabilized triplexes.

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