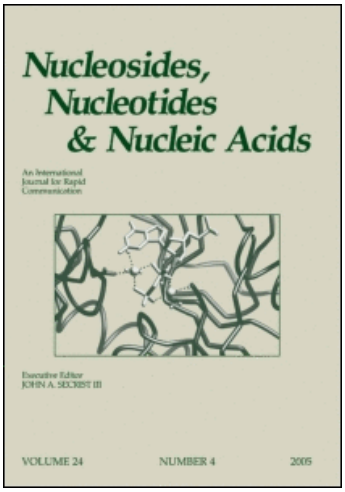


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# COMBINATION OF POLY(L-LYSINE)-GRAFT-DEXTRAN COPOLYMER AND 2′-*O*,4′-*C*-METHYLENE BRIDGED NUCLEIC ACID (2′,4′-BNA) MODIFICATION SYNERGISTICALLY STABILIZES PYRIMIDINE MOTIF TRIPLEX AT NEUTRAL PH

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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. 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] Extreme instability of the pyrimidine motif triplex at physiological pH severely limits its utility for artificial control of gene expression in vivo. Stabilization of the pyrimidine motif triplex at neutral pH is, therefore, of great importance to improve its therapeutic potential. We have previously shown that poly(L-lysine)-*graft*-dextran (PLL-*g*-Dex) copolymer (Figure 1)[2–4] and 2′-*O*,4′-*C*-methylene bridged nucleic acid (2′,4′-BNA) backbone modification of TFO (Figure 2)[5–7] increased the thermal stability of the pyrimidine

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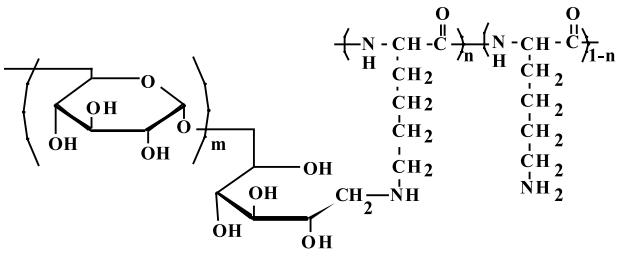

**FIGURE 1** Structural formula of PLL-*g*-Dex copolymer.

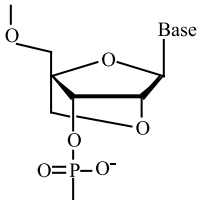


**FIGURE 2** Structural formula of 2′,4′-BNA.



motif triplex at neutral pH. In the present study, we have examined whether the combination of the two triplex-stabilizing strategies synergistically stabilizes the pyrimidine motif triplex at neutral pH.

## RESULTS AND DISCUSSION

The thermal stability of the triplex between a 15-mer TFO (Pyr15T: 5′-CTCTTCTT TTCTTTC-3′ or Pyr15BNA: 5′-*C*T*C*T*T*C*T*T*T*C*T*T*C-3′ [2′,4′-BNA modified positions are underlined]) and a 23-bp target duplex [Pur23A · Pyr23T: 5′-GCGCGAGAA-GAAAAGA AAGCCGG-3′/3′-CGCGCTCTTCTTTTCTTTCGGCC-5′] was investigated either with or without the PLL-*g*-Dex copolymer at pH 6.8 by UV melting

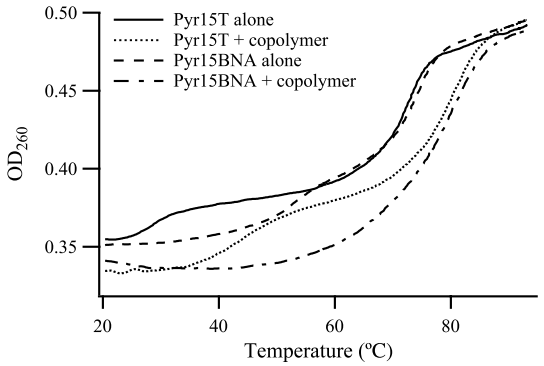


**FIGURE 3** UV melting profiles of the triplex involving the unmodified (Pyr15T) or 2′,4′-BNA modified (Pyr15BNA) TFO with or without PLL-*g*-Dex copolymer at pH 6.8.

**TABLE 1** Melting Temperatures of the Triplex Involving the Unmodified (Pyr15T) or 2′,4′-BNA Modified (Pyr15BNA) TFO With or Without PLL-*g*-Dex Copolymer at pH 6.8,[a] Obtained from UV Melting

| TFO | Copolymer | $T_{m1}$ (°C) | $T_{m2}$ (°C) |
|---|---|---|---|
| Pyr15T | – | 28.4 ± 0.7 | 72.3 ± 0.6 |
| Pyr15T | + | 44.3 ± 1.2 | 80.4 ± 0.5 |
| Pyr15BNA | – | 53.5 ± 0.3 | 73.9 ± 0.5 |
| Pyr15BNA | + | 80.4 ± 0.3[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.

(Figure 3 and Table 1). The triplex involving Pyr15T without the copolymer showed two-transition melting profile. The first transition at lower temperature, $T_{m1}$, (28.4°C) was the dissociation of the triplex to a duplex and a TFO, and the second transition at higher temperature, $T_{m2}$, (72.3°C) was the melting of the duplex. The addition of the copolymer to the triplex involving Pyr15T increased both $T_{m1}$ and $T_{m2}$ up to 44.3°C and 80.4°C, respectively. The copolymer increased the stability of both the triplex and the duplex, which was consistent with the previous studies.[2–4] On the other hand, $T_{m1}$ of the triplex involving Pyr15BNA without the copolymer (53.5°C) was significantly higher than that involving Pyr15T without the copolymer (28.4°C), although $T_{m2}$ was almost the same. The 2′,4′-BNA modification of TFO increased the stability of the triplex without changing the stability of the duplex, which was consistent with the previous results.[5–7] Finally, the triplex involving Pyr15BNA with the copolymer showed only one transition at the highest temperature, $T_m = 80.4$°C. As the magnitude in UV absorbance change at $T_m$ under this condition was almost equal to the sum of those at $T_{m1}$ and $T_{m2}$ under the above three conditions (Figure 3), the transition was identified as a direct melting of the triplex to its constituting single strand DNAs. The addition of the copolymer further increased the $T_m$ of the triplex involving Pyr15BNA by about 25°C without affecting the hyperchromicity, indicating that the copolymer and the 2′,4′-BNA modification of TFO synergistically stabilized the triplex.

We conclude that the combination of the copolymer and the 2′,4′-BNA modification of TFO synergistically increases the thermal stability of the pyrimidine motif triplex at neutral pH. Combination of different triplex-stabilizing methods could be a key strategy and may lead to progress in therapeutic applications of the antigene strategy in vivo.

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