

Synergistic stabilization of triplex by combination of comb-type cationic copolymer and oligo-N3'→P5' phosphoramidates

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ABSTRACT

In the present study, we describe rapid formation of stable pyrimidine motif triplex at physiological pH. The triplex formation was achieved by the synergistic effect of poly(L-lysine)-graft-dextran (PLL-g-Dex) copolymer and N3'→P5' phosphoramidate (PN) backbone modification of triplex-forming oligonucleotide (TFO). Either the PLL-g-Dex copolymer or the PN modification alone increased the binding constant by nearly two orders of magnitude for the triplex formation at neutral pH. The combination of both stabilizing factors that was the triplex formation with the PN TFO in the presence of the copolymer increased the binding constant by nearly four orders of magnitude. The kinetic study indicated that the copolymer increased the association rate constant, whereas the PN modification decreased the dissociation rate constant. No negative interference between these stabilizing effects was observed. The kinetically orchestrated effects in which the copolymer and the PN TFO contribute to distinct ingredients in triplex equilibrium achieved the rapid formation of the stable triplex.

INTRODUCTION

In recent years, triplex DNA has attracted considerable interest because of its possible biological function *in vivo* and

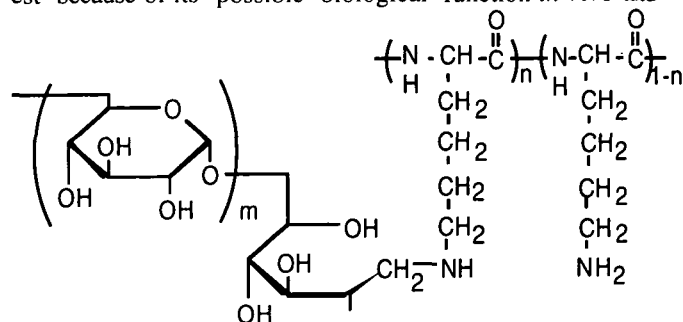


Fig. 1: Structural formula of PLL-g-Dex copolymer.

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). 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. However, stabilization of the pyrimidine motif triplex at neutral pH is quite necessary for its applicability as an anti-gene drug *in vivo*. We have previously shown that poly(L-lysine)-graft-dextran (PLL-g-Dex) copolymer (Fig. 1) increased the binding constant of the pyrimidine motif triplex formation at neutral pH (2-4). Our group and others have also shown that N3'→P5' phosphoramidate (PN) backbone modification of TFO (Fig. 2) increased the thermal stability of the pyrimidine motif triplex and the binding constant of the pyrimidine motif triplex formation at neutral pH (5-7). Here, we have tested whether the stability of the pyrimidine motif triplex with PN TFO can be further increased by the addition of the copolymer. We have found that the combination of the copolymer and the PN modification increased the binding constant by nearly four orders of magnitude for the pyrimidine motif triplex formation at neutral pH.

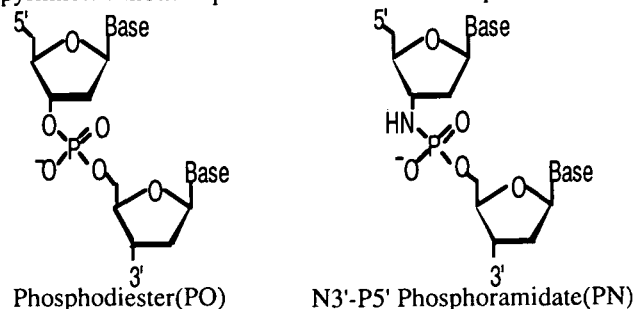


Fig. 2: Structural formulas of oligonucleotides.

Table 1: Kinetic parameters for the triplex formation between a 15-mer TFO (Pyr15T or Pyr15NP) and a 23-bp target duplex (Pur23A•Pyr23T) at 25 °C and pH 6.8^a with or without 0.038 mM PLL-g-Dex copolymer, obtained from IAsys

TFO	PLL-g-Dex copolymer	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 x 10 ²	1.0	1.17 x 10 ⁻²	1.0	5.41 x 10 ⁴	1.0
Pyr15T	+	2.88 x 10 ⁴	45.6	7.8 x 10 ⁻³	0.66	3.69 x 10 ⁶	68.2
Pyr15NP	-	1.19 x 10 ³	1.89	3.36 x 10 ⁻⁴	0.029	3.54 x 10 ⁶	65.4
Pyr15NP	+	5.27 x 10 ⁴	83.5	2.26 x 10 ⁻⁴	0.019	2.33 x 10 ⁸	4310

^a10 mM sodium cacodylate-cacodylic acid, 200 mM sodium chloride and 20 mM magnesium chloride (pH 6.8).

MATERIALS AND METHODS

We synthesized a 15-mer TFO, Pyr15T: 5'-CTCTTCTTTTCTTTC-3', and complementary 23-mer DNA oligonucleotides, Pur23A: 5'-GCGCGAGAAGAAAAGAAAGCCGG-3' and Pyr23T: 5'-CCGGCTTCTTTTCTTCTCGCGC-3', on a DNA synthesizer and purified them with a reverse-phase HPLC. A modified TFO with the same sequence as Pyr15T but with PN backbone modification, Pyr15NP, were synthesized as described previously (5). The PLL-g-Dex copolymer was prepared by a reductive amination reaction between poly(L-lysine) and Dextran T-10, as described previously (2). 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 (4, 7, 8, 9).

RESULTS AND DISCUSSION

Table 1 summarizes the kinetic parameters of the triplex formation between a 15-mer TFO (Pyr15T or Pyr15NP) and a 23-bp target duplex (Pur23A•Pyr23T) at 25 °C and pH 6.8 with or without PLL-g-Dex copolymer, obtained from IAsys. Either the copolymer or the PN modification increased K_a by nearly 70 times. However, their kinetic effects were obviously different from each other. The copolymer increased k_{assoc} by about 50 times, whereas it decreased k_{dissoc} by only 1.5 times. Thus, acceleration of the triplex formation is the major effect of the copolymer to increase K_a . In contrast to the copolymer, the PN modification decreased k_{dissoc} by about 35 times, while it moderately increased k_{assoc} by no more than 2 times. Therefore, maintaining the triplex structure is the major effect of the PN modification to increase K_a . Unexpectedly, the combination of the copolymer and the PN modification resulted in more than 4000 times increase in K_a . The increase in K_a results mainly from the increase in k_{assoc} by the copolymer and the decrease in k_{dissoc} by the PN modification. Moreover, by comparing the kinetic parameters, we noticed that k_{assoc} and k_{dissoc} obtained for Pyr15NP with the copolymer closely coincided with the products of the values that were individually obtained for either Pyr15T with the copolymer or Pyr15NP alone. The result clearly demonstrated that neither interference nor negative cooperation between their kinetic effects was involved in their synergistic stabilization mechanism.

Consequently, the kinetically harmonized effects in which the PLL-g-Dex copolymer and the PN modification contribute to distinct ingredients in triplex equilibrium permits the rapid formation of sturdy pyrimidine motif triplex at physiological pH, which has not yet been achieved. To our knowledge, this is the first demonstration of orchestrating different strategies for triplex stabilization. Recently, PN TFO has been demonstrated to be effective to modulate gene expression through triplex-mediated inhibition of transcription elongation *in vivo* (10). Further increase in k_{assoc} as well as K_a of PN TFO by the PLL-g-Dex copolymer could greatly enhance its therapeutic potential. We conclude that thermodynamic and kinetic analyses enable us to select a rational combination of different stabilizing strategies for achieving high triplex stability at physiological pH, which is prerequisite for advancing triplex-mediated DNA targeting *in vivo*.

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