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Alternate stranded triplex formation of chimeric DNA composed of tandem α- and β-anomeric strands

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A chimeric oligoDNA composed of a natural β-anomeric oligonucleotide portion and an unnatural α-anomeric oligonucleotide portion forms an alternate stranded triplex possessing enhanced thermal stability compared to the triplexes composed of the parental oligomers.

Formation of a triple-helical DNA through the binding of a third strand DNA to a double-stranded (ds) genomic DNA has been attracting great interest as a possible new therapeutic method, because it would impede replication and/or transcription of the specific gene (anti-gene methodology). Thus, a large number of papers have been published dealing with a triple helix-forming oligonucleotide (TFO).[1]

Among these, a TFO proposed by Dervan *et al*.[2] has a unique feature. The TFO consists of two independent DNA polythymidylates coupled by 1,2-dideoxyribose through a 3′-3′ phosphodiester linkage. Thus, the oligomer has 5′-to-5′ orientation and simultaneously binds to complementary purine tracts in the 5′-(Pu)m(Py)n-3′ and 3′-(Py)m(Pu)n-5 region of dsDNA to form a so-called alternate stranded triplex in a parallel orientation manner. The resulting alternate stranded triplex has an increased thermal stability. Since then, a number of analogous TFOs utilizing either 3′-3′ or 5′-5′ linked oligonucleotides,[3] or both CT blocks (forming a parallel triplex) and GT or GA blocks (forming an anti-parallel triplex) have been reported.[4] These TFOs, however, are composed of natural β-anomeric deoxyribonucleotide units. Meanwhile, non-natural α-anomeric oligodeoxypyrimidylate containing both thymidine and deoxycytidine residues binds to a purine tract of dsDNA in an anti-parallel orientation.[5]

Aiming to create a new class of TFO capable of forming an alternate stranded triple helix, we have synthesized α-β chimeric oligoDNA (**ODN-1**)[6] in which the mononucleotide units are connected through natural 3′-5′ phosphodiester linkages although the 3′-half of **ODN-1** exclusively consists of α-anomeric polypyrimidylate and the remaining portion consists of β-anomeric polypyrimidylate. Therefore, the 3′-half of **ODN-1** would bind to the purine tract of **ODN-2** in an anti-parallel orientation while the remaining β-DNA portion, except the 5′-CCAA-3′ portion which serves as a 'linker' connecting the α-DNA and β-DNA portions, would bind to another purine tract of **ODN-3** in a parallel orientation (Fig. 1). Although the possibility of the alternate triplex formation with such chimeric DNA was once mentioned,[1c] no experimental report has been presented before. Here, we present the first example that the chimeric DNA actually forms a stable alternate strand triplex with the targeted dsDNA.

ODN-1 was synthesized using an automated DNA synthesizer (ABI 381-A) on a 1 μmol scale, starting from CPG-bound α-deoxynucleoside which was prepared according to a known procedure.[7] Several deoxycytidine residues in **ODN-1** were substituted with its 5-methyl analog to secure the triplex-forming ability under near neutral conditions.[8] The introduction of 5-methyl deoxycytidine residues to the oligomer was achieved by the reported procedure.[9] After the assembly, the CPG-bound oligomer was treated with conc. ammonia[9] and purified by reversed-phase HPLC, detritylation, ethanol precipitation and Sephadex G-25 gel filtration to give **ODN-1** in satisfactory yield (28.7%).†

The melting temperature (T_m) of the complex composed of **ODN-1** and the dsDNA was examined by a UV-melting experiment (Fig. 2). The T_m of the complexes composed of the independently prepared α-DNA portion (α-**11mer**) or the β-DNA portion (β-**11mer**) alone with the dsDNA were also measured as controls. In Fig. 2, higher transitions are due to the dissociation of the duplex (**ODN-2** + **ODN-3**) into the single strands.‡ The lower transitions may correspond to the dissociation of the triplex into the duplex and the third strand. The data suggest that the formation of the triplex actually occurred upon mixing **ODN-1** with the dsDNA under the conditions we used. The estimated T_m value of the triplex containing **ODN-1** was, however, considerably higher than those of the triplexes containing the parental α-**11mer** or β-**11mer** alone (Table 1). The complex composed of the duplex and the mixture of both the parental α-**11mer** and β-**11mer** (1 : 1) under the same conditions as above gave almost the same melting profile as the other complexes (two-phase transition). However, the putative T_m value for the triplex in this case was far below (26.1 °C, approximately) than that of the complex containing **ODN-1** (38.5 °C, see Table 1). These results strongly indicate that the current chimeric DNA forms an alternate stranded triplex in

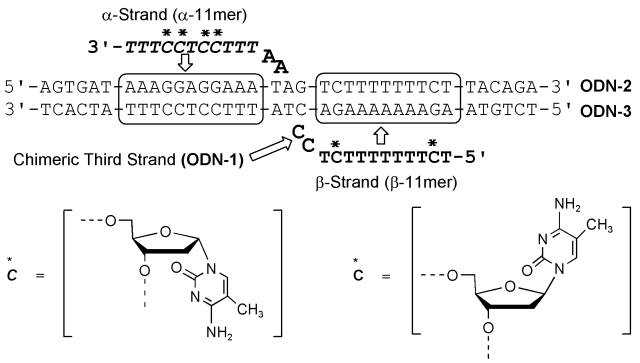

Fig. 1 The sequence and the structure of α-β chimeric DNA (**ODN-1**). In **ODN-1**, italic letters represent α-anomeric deoxynucleotide and roman letters represent β-anomeric deoxynucleotide, respectively.

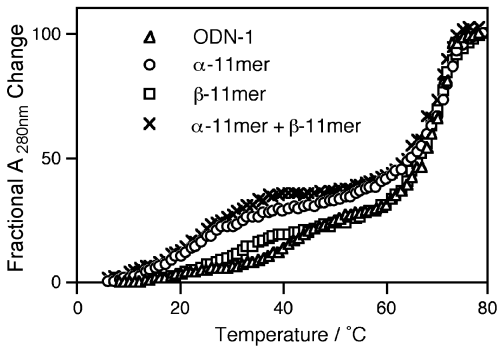

Fig. 2 UV melting profiles of the triplexes.

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Table 1 T_m values of the triplexes and the duplexes

TFO	Triplex T_m (°C)	Duplex T_m (°C)
ODN-1	38.5	70.0
α-11mer	26.2	70.3
β-11mer	27.2	70.2
α-11mer + β-11mer	26.1	70.3

T_m values were determined by computer fit of the first derivative of the absorbance with respect to 1/*T*.

which the longer region of the dsDNA is involved for triplex formation compared to the cases using the parental 11mer alone or the mixture of those as the TFO. It should be noted that a single transition step for the dissociation of an alternate stranded triplex to a duplex and a third strand was observed previously.[4h]

Next, a DNase 1 footprinting experiment was performed to analyze the structure of the triplex. The dsDNA containing ^{32}P radio-labeled **ODN-2** at its 5′-terminus was digested by DNase 1 in the presence or the absence of **ODN-1** at 5 °C and the products were analyzed by denaturing 20% polyacrylamide gel electrophoresis. As shown in Fig.3, the dsDNA was thoroughly digested by the nuclease in the absence of **ODN-1** to give numerous lengths of products (lane 3). On the other hand, the expected triplex-forming regions in the dsDNA indicated in Fig. 1 and Fig. 3 (boxed-regions) were thoroughly protected from the digestion in the presence of **ODN-1** (lane 6). The protected regions in lane 6 nicely correspond to the triplex forming regions in lanes 4 and 5 in which the parental α-**11mer** and β-**11mer** were used as the TFO, respectively. These results along with results obtained from the T_m experiment clearly indicate

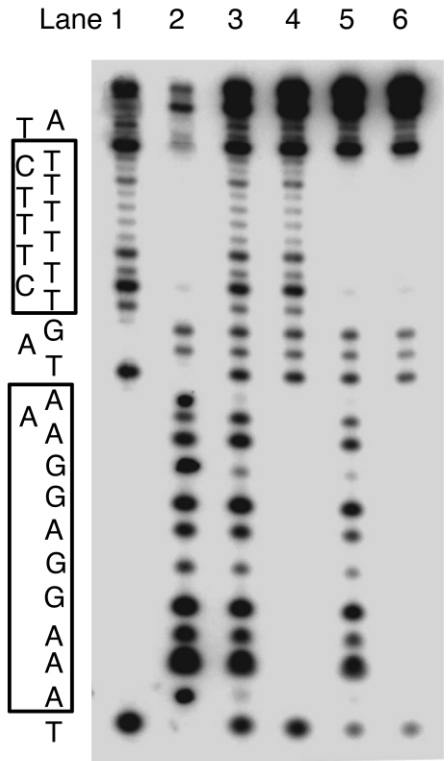

Fig. 3 Results of DNase 1 footprinting experiment. Lanes: 1, T+C sequence (Maxam-Gilbert); 2, G+A sequence (Maxam-Gilbert); 3, DNase 1 digestion of the dsDNA; 4, DNase 1 digestion of the dsDNA + α-**11mer**; 5, DNase 1 digestion of the dsDNA + β-**11mer**; 6, DNase 1 digestion of the dsDNA + **ODN-1**. **ODN-1**·ODN-2 duplex (0.3 μM) was mixed with appropriate TFO (15 μM) in sodium phosphate buffer (pH 6.4) containing 100 mM NaCl, 0.5 mM spermine and 5 mM $MgCl_2$. The mixture was digested with DNase 1(7.0 units) at 10 °C for 30 min.

that **ODN-1** forms an alternate stranded triplex with the dsDNA at the expected regions. Also, the linker portion of the chimera (5′-CCAA-3′) has enough length and flexibility to secure the desired triplex formation, at least in this case.

In conclusion, we have successfully demonstrated the first example of an α-β chimeric DNA to form a stable alternate stranded triplex that can serve as a new class of TFO. The current chimeric DNA may have an advantage over the previously reported TFOs. For example, the chimera is highly resistant toward the action of 3′-exonuclease as like α-DNA.[10]§ The nuclease has been suggested to play an important role in degradation of oligonucleotides in living systems.[6c,11] Works to enhance the thermal stability of the triplex by conjugating the chimera with an appropriate functional molecule as well as the optimization of the length of linker portion are now under way in our laboratory and will be reported elsewhere.

Notes and references

† The following fragments were detected; *m/z* 982.6 $[M + 2Na]^{8-}$ (calcd. 982.7), 1126.4 $[M + 3Na]^{7-}$ (calcd. 1126.3), 1318.0 $[M + 4Na]^{6-}$ (calcd. 1317.9), 1982.5 $[M + 5Na]^{4-}$ (calcd. 1982.8).

‡ ODN-2·ODN-3 duplex (1.5 μM) was mixed with appropriate TFO (1.5 μM) in sodium cacodylate (pH 6.4) containing 100 mM NaCl, 0.5 mM spermine and 10 mM $MgCl_2$. The temperature was raised at 0.1 °C min^{-1} and thermally induced transition of each mixture was monitored at 280 nm.

§ In the experiment using snake venom phosphodiesterase (0.1 unit) the parental β-**11mer** (1 mM) was fully degraded within 15 min. while the α-**11mer** and **ODN-1** were not affected at all under the same conditions.

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