Promotion of stable triplex formation by partial incorporation of 2′,5′-phosphodiester linkages into triplex-forming oligonucleotides†

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Pentadecamer homopyrimidine oligonucleotides containing three or more 2′,5′-phosphodiester linkages in different modes were prepared and used to evaluate the ability as a triplex-forming oligonucleotide (TFO), and it was found that discontinuous replacement of the 3′,5′-phosphodiester linkages in TFO by 2′,5′-linkages significantly stabilizes parallel-motif triplexes.

To resolve the question why nucleic acids carrying genetic information exclusively have a 3′,5′-phosphodiester linkage, many studies have been carried out to date.[1] Lariat RNA[2] and 2–5A[3] are examples of 2′,5′-linked nucleic acids (2′,5′-NAs) existing in a living cell. These 2′,5′-NAs perform important functions in RNA splicing and in interferon-treated cells, respectively, but not in storage and transfer of genetic information. The 2′,5′-NAs are able to form a duplex by themselves[4,5] or with the 3′,5′-NA complements;[6–8] however, the thermal stability of these duplexes is lower than that of the corresponding 3′,5′-linked duplexes. The difference in thermal stability between the 2′,5′-linked DNA (2′,5′-DNA) duplex and the 3′,5′-linked DNA (3′,5′-DNA) duplex would be one reason why the 3′,5′-linkage was selected instead of the 2′,5′-linkage (Fig. 1).

Association of the triplex-forming oligonucleotide (TFO) with 3′,5′-DNA duplexes under physiological pH and salt conditions is very important from the viewpoint of biomedical applications[9] and understanding of the triplex structure in detail. The ability of 2′,5′-RNA to hybridize with 3′,5′-DNA duplexes was evaluated by Damha and Noronha,[10] and the 2′,5′-linked TFO showed a slight decrease in triplex-forming ability. To the best of our knowledge, there has been no report that the 2′,5′-linked TFO enhances the binding affinity with 3′,5′-DNA duplexes. Here we would like to demonstrate that the TFOs partially including 2′,5′-linkages form stable parallel-motif triplexes with the target 3′,5′-duplex under near physiological conditions.

We prepared the 15-mer TFOs containing 2′,5′-DNA moieties according to the literature.[11,12] The sequences of the TFOs and target duplexes used in this study are shown in Fig. 2. UV-melting experiments were performed in 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM potassium chloride (conditions A) to investigate the triplex-forming ability of the TFOs. At first, we observed that the TFO **2** containing three successive 2′,5′-linkages formed a triplex structure with a 21-bp target duplex **8**; however, the complex was less stable than the parent triplex **1·8** (Fig. 3). This result is consistent with the previous report on 2′,5′-RNA.[10] Surprisingly, the TFO **3** including three 2′,5′-linkages not continuously but at every third residue showed remarkable stabilization of the triplex (Fig. 3). The difference in melting temperature (T_m) between the triplexes **3·8** and **2·8** was 11 °C. To confirm this result, the thermal stability of the triplexes comprising other TFOs **4–7** which have four or more 2′,5′-linkages was also evaluated (Table 1). As a result, all the TFOs including the 2′,5′-linkages at every two or more residues significantly enhanced the triplex stability. An increase of 2.0–3.0 °C per 2′,5′-linkage in T_m value was observed when compared to the parent triplex **1·8**. Similar results were obtained in 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM potassium chloride and 10 mM magnesium chloride (conditions B). Thus, we first demonstrate that an appropriate 2′,5′-DNA modification of TFOs promotes the parallel-motif triplex formation under near physiological conditions.

Next, the helical structure of the triplexes was evaluated by CD measurements (Fig. 4). All the CD spectra of the triplexes **4·9**, **6·9** and **1·9** showed a negative band at 210 nm which is characteristics of the triplex structure,[13,14] while the spectrum of duplex DNA or

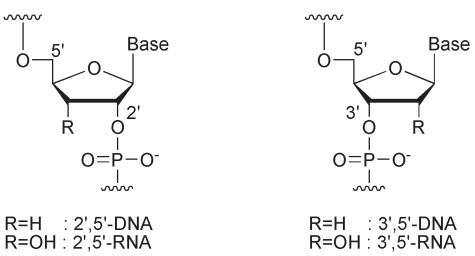

Fig. 1 Structures of 2′,5′- and 3′,5′-linked nucleic acids.

TFOs
5′-d(TTTTT^{m}CTTT^{m}CT^{m}CT^{m}CT)-3′ (**1**)
5′-d(TTTTT^{m}C**T**T**T**^{m}CT^{m}CT^{m}CT)-3′ (**2**)
5′-d(TTTT**T**^{m}CT**T**T^{m}C**T**^{m}CT^{m}CT)-3′ (**3**)
5′-d(T**T**TT**T**^{m}CT**T**T^{m}C**T**^{m}CT^{m}CT)-3′ (**4**)
5′-d(**T**TTTT**T**^{m}CTT**T**^{m}CT^{m}C**T**^{m}CT)-3′ (**5**)
5′-d(TT**T**T**T**^{m}C**T**T**T**^{m}C**T**^{m}CT^{m}CT)-3′ (**6**)
5′-d(**T**T**T**T**T**^{m}C**T**T**T**^{m}C**T**^{m}C**T**^{m}CT)-3′ (**7**)

Target duplexes
5′-d(GCT*AAAAAGAAAGAGAGA*TCG)-3′ (**8**)
3′-d(CGA*TTTTTCTTTCTCTCT*AGC)-5′

5′-d(GCTGCT*AAAAAGAAAGAGAGA*TCGTCG)-3′ (**9**)
3′-d(CGACGA*TTTTTCTTTCTCTCT*AGCAGC)-5′

Fig. 2 Sequences of the TFOs **1–7** and the target duplexes **8** and **9** used in this study. **T**: 2′,5′-linked 3′-deoxythymidine. ^{m}C: 2′-deoxy-5-methylcytidine. TFO binding sites in the target duplexes **8** and **9** are shown in italic.

† Electronic supplementary information (ESI) available: experimental details and T_m profiles. See http://www.rsc.org/suppdata/cc/b4/b417688j/

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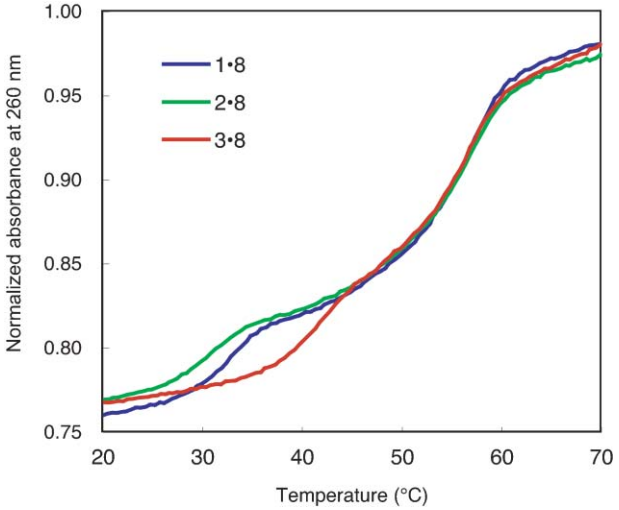

Fig. 3 Normalized UV melting curves of the triplexes **1·8** (blue), **2·8** (light green) and **3·8** (red) in 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM potassium chloride (conditions A).

Table 1 Melting temperatures (T_ms) of the triplexes[a]

	T_m (ΔT_m/modification)/°C	
TFO	Target duplex **8** Conditions A[b]	Target duplex **9** Conditions B[c]
1	33	43
2	31 (−0.7)	40 (−1.0)
3	42 (+3.0)	51 (+2.7)
4	41 (+2.0)	52 (+2.5)
5	44 (+2.8)	53 (+2.5)
6	46 (+2.6)	54 (+2.0)
7	47 (+2.0)	57 (+2.0)

The melting temperatures for dissociation of the target duplexes **8** and **9** were 57 and 71 °C, respectively. [a] UV melting profiles were measured at a scan rate of 0.5 °C min^{-1} with detection at 260 nm. The melting temperatures were obtained as the maxima of the first derivative of the melting curves. [b] Conditions A: 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM potassium chloride. [c] Conditions B: conditions A + 10 mM magnesium chloride.

TFOs alone exhibited no negative band at this region (Fig. 4(A)–(C)). Furthermore, the spectral pattern of the triplexes **4·9** and **6·9** is quite similar to that of the parent triplex **1·9** (Fig. 4(D)). Thus, partial incorporation of 2′,5′-linkages into TFOs promotes stable triplex formation without any disordering of the overall triplex structure, though the number of backbone bonds in the 2′,5′-NA chain is different from that in the 3′,5′-NA.

The difference between the number of backbone bonds in the 2′,5′- and 3′,5′-NAs will result in change in the distance between the neighboring phosphorous atoms (P···P) in TFOs, and eventually it may cause a structural disorder of the triplex. However, no such unusual triplex structure was observed in this study (Fig. 4). The P···P distance in the 2′,5′- or 3′,5′-NAs also depends on the sugar conformation, and it is known that a "compact" N-type (C3′-*endo*) sugar conformation (P···P = 5.9 Å) in the 3′,5′-NAs corresponds to an S-type (C2′-*endo*) conformation (P···P = 5.9 Å) in the 2′,5′-NAs.[15] Similarly an "extended" S-type (C2′-*endo*) sugar puckering (P···P = 7.0 Å) in the 3′,5′-NAs corresponds to an N-type (C3′-*endo*) conformation (P···P = 7.5 Å)

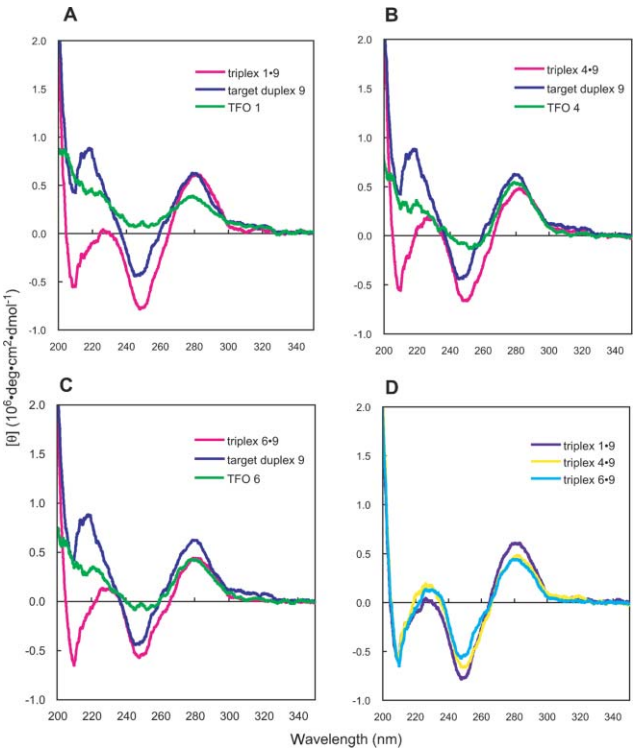

Fig. 4 CD spectra of the triplex (magenta), target duplex (blue) and TFO (green) (A)–(C), and comparison of the spectrum of the triplexes **1·9** (purple), **4·9** (yellow) and **6·9** (cyan) (D). The CD spectra were measured under conditions B.

in the 2′,5′-NAs.[15] In the 3′,5′-NAs, the "compact" N-type sugar conformation of TFO is likely to be suitable for formation of a stable triplex structure, which is supported by several studies on the nucleic acid analogues having N-type sugar conformation such as 2′,4′-BNA/LNA,[16,17] ENA,[18] and oligonucleotide N3′→P5′ phosphoramidates.[19] Among these previous studies, it is noteworthy that partially modified 2′,4′-BNA-TFOs significantly enhanced the triplex stability,[16,17] while the fully and continuously modified one failed to form a stable triplex.[16] Considering these results, the 2′,5′-DNA moieties in TFOs **3–7** are likely to have a "compact" S-conformation, and the presence of the "compact" conformation at every two or more residues, not at continuous residues, in TFOs may contribute to the triplex stabilization.

In conclusion, we have found that the pyrimidine-motif TFO partially including 2′,5′-linkages forms a very stable triplex under near physiological conditions. The overall helical structure of the obtained triplexes was similar to that of the unmodified triplex. These results would be helpful not only for development of an antigene molecule but also to understanding the detailed structure of the triplex. Further studies on the relationship between the sugar puckering and the triplex-forming ability of the 2′,5′-linked TFOs are now in progress.

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