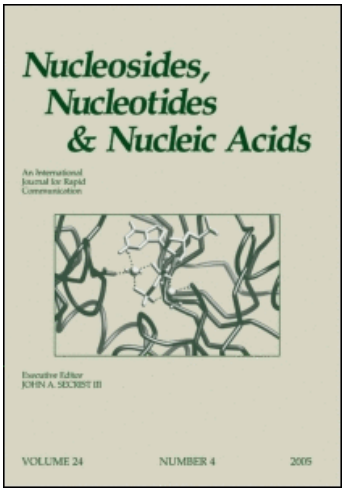


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# Presence of 2',5'-Linkages in a Homopyrimidine DNA Oligonucleotide Promotes Stable Triplex Formation Under Physiological Conditions

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# PRESENCE OF 2′,5′-LINKAGES IN A HOMOPYRIMIDINE DNA OLIGONUCLEOTIDE PROMOTES STABLE TRIPLEX FORMATION UNDER PHYSIOLOGICAL CONDITIONS

**Satoshi Obika, Akiko Hiroto, Osamu Nakagawa, and Takeshi Imanishi**
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▫ *We prepared 15-mer homopyrimidine oligonucleotides containing three or four 2′,5′-linked DNA units, and their ability as a triplex-forming oligonucleotide (TFO) was analyzed in detail. UV melting experiments showed that replacement of a 3′,5′-linkage by a 2′,5′-linkage at every third or fourth residue in TFO significantly promoted stable triplex formation under physiological conditions.*

**Keywords** Triplex, 2′,5′-Phosphdiester Linkage, Melting Temperature



## INTRODUCTION

Nucleic acids having 2′,5′-phosphodiester linkages instead of the 3′,5′-linkages are known to have the following features (Figure 1). The 2′,5′-linked nucleic acids play an important role in RNA splicing[1] and in interferon-treated cells.[2] The self-complementary 2′,5′-linked oligonucleotides (2′,5′-ONs) formed antiparallel duplex structures, but with reduced stability compared to their 3′,5′-linked isomers.[3] Very interestingly, it was also found that the 2′,5′-ONs formed a stable duplex with their RNA complements but not with DNA.[4–6]

Stable triplex formation between the triplex-forming oligonucleotide (TFO) and the target double-stranded DNA is fundamental to the antigene strategy to regulate gene expression in a living cell. The 2′,5′-ON was known to hybridize with a 3′,5′-linked DNA duplex to form a triplex structure; however, the stability of the triplex was lower than that of the corresponding 3′,5′-linked DNA triplex.[7] Here we would like to describe that appropriate incorporation of the 2′,5′-linkages into TFOs significantly enhances triplex stability under physiological conditions.

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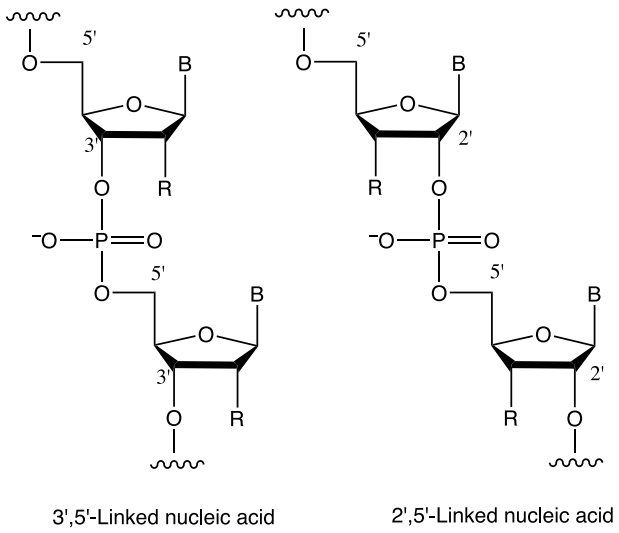


**FIGURE 1** Structure of 3′,5′- and 2′,5′-linked nucleic acids.



## MATERIALS AND METHODS

We synthesized a 15-mer homopyrimidine TFO, **TFO1**: 5′-TTTTT$^{m}$CTTT$^{m}$-CT$^{m}$CT$^{m}$CT-3′ ($^{m}$C: 2′-deoxy-5-methylcytidine), and complementary 21-mer ONs, **Pu21**: 5′-GCTAAAAAGAAAGAGAGATCG-3′ and **Py21**: 5′-CGATCTCTC-TTTCTTTTTAGC-3′, on a DNA synthesizer and purified them with a reverse-phase HPLC. TFOs containing 2′,5′-phosphodiester linkages, **TFO2**: 5′-TTT-TT$^{m}$C***TTT***$^{m}$CT$^{m}$CT$^{m}$CT-3′, **TFO3**: 5′-TTTT***T***$^{m}$CT***T***T$^{m}$C***T***$^{m}$CT$^{m}$CT-3′, **TFO4**: 5′-T***T***TT***T***$^{m}$CT***T***T$^{m}$C***T***$^{m}$CT$^{m}$CT-3′ and **TFO5**: 5′-***T***TTT***T***$^{m}$CTT***T***$^{m}$-CT$^{m}$C***T***$^{m}$CT-3′ (***T***: 2′,5′-DNA-T), were prepared according to the literature.[8,9] UV melting experiments were carried out on a Beckman DU650 spectrophotometer equipped with $T_m$ analysis accessory using quartz cuvettes of 1 cm optical path length. Samples were dissolved at 1.5 μM strand concentration in 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM potassium chloride. The temperature of the cell holder was increased from 10 to 85°C at a rate of 0.5°C/min, and the absorbance at 260 nm was recorded every minute.

## RESULTS AND DISCUSSION

Triplex-forming ability of the TFOs was evaluated by UV melting experiments. Melting temperatures ($T_m$s) of the triplexes are summarized in Table 1. The **TFO2** having three successive 2′,5′-linkages formed a triplex structure with a 21-bp DNA target **Pu21**·**Py21**; however, the complex was less stable than the parent triplex comprising the **TFO1** and the **Pu21**·**Py21**. This result is consistent with the previous studies on the 2′,5′-linked RNA.[7] Surprisingly, the **TFO3** containing three 2′,5′-linkages, not continuous but at every third residue, formed very stable

**TABLE 1** Melting Temperatures ($T_m$s) of the Triplexes Between a 15-mer TFO (**TFO1**, **TFO2**, **TFO3**, **TFO4** or **TFO5**) and a 21-bp Target Duplex (**Pu21**·**Py21**)[a]

| TFOs | $T_m$ ($\Delta T_m$/modification) (°C) |
|---|---|
| **TFO1** | 33 |
| **TFO2** | 31 (−0.7) |
| **TFO3** | 42 (+3.0) |
| **TFO4** | 41 (+2.0) |
| **TFO5** | 44 (+2.8) |

[a]Conditions: See Materials and Methods.

triplexes under physiological conditions. Furthermore, the **TFO4** and **TFO5** which include four 2′,5′-linkages also showed remarkable stabilization of the triplexes. An increase of 2.0–3.0°C per 2′,5′-linkage in $T_m$ value was observed when compared to the parent triplex **TFO1**·**Pu21**·**Py21**. Thus, we have first demonstrated that a partial incorporation of the 2′,5′-linkages into TFOs promotes a stable triplex formation.

The conformation of a sugar moiety in TFOs has a considerable effect on the stability of a triplex. In the 3′,5′-linked triplexes, the N-type sugar conformation of TFOs is likely to be suitable for formation of a stable complex.[10] Yathindra et al. proposed that a "compact" N-type sugar conformation in the 3′,5′-ONs corresponds to an S-type conformation in the 2′,5′-ONs, while an "extended" S-type conformation in the 3′,5′-ONs corresponds to an N-type conformation in the 2′,5′-ONs.[11] Considering these points, the 2′,5′-linked DNA moieties in **TFO3**, **TFO4** and **TFO5** may have a "compact" S-type sugar conformation. Further studies on the relationship between the sugar conformation and the triplex-forming ability are now in progress.



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