Synthesis and Properties of Novel Oligodeoxynucleotides Containing 6,3′-Methanodeoxyuridine

Tomohisa Moriguchi, Fumisada Yamato, and Kazuo Shinozuka*
Graduate School of Engineering, Gunma University, 1-5-1 Tenjincho, Kiryu 376-8615

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This paper deals with the synthesis and properties of novel oligodeoxynucleotide containing 6,3′-methanodeoxyuridine which is connected between C-6 of pyrimidine base and C-3′ of deoxyribose by a methylene group. The modified base was successfully introduced into the oligodeoxynucleotide by the standard method. The modified oligodeoxynucleotide formed the less stable duplex with both complementary DNA and RNA. The CD spectra indicated that the duplex formed the noncanonical duplex structure distinct from the A-form or B-form structure.

Numerous numbers of modified oligonucleotides that is applicable for the gene therapeutics and the gene diagnosis have been reported.[1] For application to the antisense therapeutics, one of the main requirements for the modified oligonucleotides is the ability to the formation of the physiologically stable duplex with complementary RNA.[2] In general, RNA duplex forms the A-type duplex structure, and the sugar puckering of the deoxyribose constrained to C-3′ endo form at that time. Therefore, when the modified nucleoside that constrained to the C-3′ endo puckering in advance was introduced into oligonucleotides, the oligonucleotide can form the thermodynamically stable duplex with the complementary RNA. Many numbers of the modified nucleosides that satisfy the conformational requirement were reported up to date by several groups,[3–6] and the oligonucleotides that are consisted of such modified nucleosides are expected to become the next generation antisense materials.[7]

On the other hand, the glycosidic torsion angle of deoxyribose was also constrained to anti conformer when the duplex was formed, in spite of the free rotation at the single-stranded state. Therefore, if the glycosidic torsion angle and the sugar puckering were constrained to anti and C3′-endo simultaneously, the more thermodynamically stable duplex was expected to construct by the introduction of such a modified nucleoside. Previously, Ueda et al. reported the cyclonucleoside that the C-3′ of ribose and C-6 of pyrimidine base was bridged by the methylene linker.[8] The sugar puckering of this cyclonucleoside was constrained to the C-3′ conformer by the methylene linker. In this paper, we reported the synthesis and properties of the novel oligonucleotide containing this cyclonucleoside.

The cyclonucleoside, 2′-deoxy-6,3′-methanouridine was prepared by the coupling of the 3-ketosugar and the 6-lithiated 6-methyluracil derivative according to the previous report.[8] The 5′-OH of 2′-deoxy-6,3′-methanouridine was protected by the DMTr group in 33% yield. The phosphitylation of 5′-DMTr derivative was carried out by the standard method and needed longer reaction time (1 h) than the canonical deoxynucleoside. The desired phosphoramidite derivative was obtained in 40% yield. The ^{31}P NMR analysis showed the characteristic peaks that the chemical shifts of the diastereomeric two peaks slightly shifted to the high magnetic field. This high magnetic field shift seemed to be characteristic for the phosphoramidite derivatives of the tertiary alcohol reported by the other group.[9]

The synthesis of modified oligonucleotide containing the cyclonucleoside was performed on an automated synthesizer. The cyclonucleoside was introduced into the central position of the 15-mer oligonucleotide (Figure 1). The coupling time for the cyclonucleoside was elongated to 360 s for the sufficient reaction, however, the coupling yield lowered (90%).

The structure of the modified oligonucleotide (ODN-1) was elucidated by the treatment of the appropriate nuclease and phosphatase to give the corresponding nucleoside. After treatment with snake venom phosphodiesterase (SVPDE, 3′-exonuclease) and alkaline phosphatase (AP), the HPLC analysis showed the four peaks (Figure 2A). However, the area of each peak is in conflict with the ratio of the corresponding nucleoside, the number of deoxycytidine calculated from the ratio of the peak area was fewer than one's expectation. The fourth peak except for the canonical nucleoside was assigned to the dimer, dCpX. Therefore, further treatment with nuclease P1 (endonuclease) eliminated this fourth peak, however, no new peak that

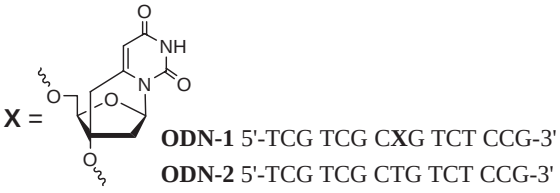

Figure 1. The structure and the sequenceof the novel ODN in this study.

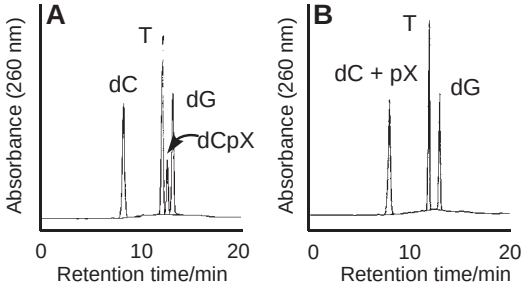

Figure 2. HPLC profiles of the hydrolysate product from the treatment of ODN-1 using snake venom phosphodiesterase (SVPDE), nuclease P1, and alkaline phosphatase (AP). (A) ODN-1 was treated with SVPDE, and AP. (B) Further treatment of the sample of 2A with NP-1. The analysis was carried out by reversed phase HPLC on Wakosil 5C-18 column (4 mmf × 250 mm) using 0.1 M TEAA (pH 7.0) with a linear gradient of MeCN.

Table 1. Melting temperatures of the duplexes containing the modified oligonucleotides[a]

	cDNA-1[b]	cDNA-2[b]	cDNA-3[b]	cRNA-1[b]
ODN-1	53.6	53.4	42.3	55.0
ODN-2	64.0	53.2	53.8	65.8
ΔT_m	−10.4	+0.2	−11.5	−10.8

[a]The UV-melting curves were measured at 260 nm with oligonucleotide concentration of 2.5 mM in sodium phosphate buffer (10 mM, pH 7.2) containing 100 mM NaCl. The T_m values were obatained from first deviatives of the melting curves. [b]cDNA-1 and cRNA-1: 5′-CGG AGA CAG CGA CGA-3′, cDNA-2: 5′-CGG AGA CGG CGA CGA-3′, cDNA-3: 5′-CGG AGA CAG CGG CGA-3′.

was expected to be assigned to pX was observed (Figure 2B). Furthermore, the number of deoxycytidine calculated from the ratio of the peak area was more than one's expectation. Therefore, deoxycytidine and the cyclonucleoside showed the same retention time at these analytic conditions. Consequently, 6,3′-methanodeoxyuridine showed the resistance at the 5′-phosphodiester linkage against the action of the 3′-exonuclease, snake venom phosphodiesterase, and this fact about the nuclease resistance is consistent with the previous reports[10,11] that the glycosidic bond-restricted nucleoside promoted the same resistance.

The stability of the duplexes formed by ODN-1 or the corresponding unmodified DNA, ODN-2, and their complementary or one base mismatched DNA or RNA were analyzed by the UV-melting experiments and Table 1 shows the obtained T_m values. cDNA-1 and cRNA-1 had the fully complementary sequence to ODN-1, 2, and cDNA-2 and cDNA-3 had one base mismatched sequences at the central and 4th position from the 3′-terminus, respectively. The T_m values of the modified duplexes were dramatically lower than those of the unmodified both DNA and RNA. Therefore, the introduced cyclonucleoside drastically destabilized the duplexes formed with both DNA and RNA at a similar magnitude. However, the displacement of the base at the complementary position of the cyclonucleoside from A to G showed no significant difference in thermal stability, in spite of the drastic destabilization in the case of ODN-2. It seemed that the cyclonucleoside had no interaction with the complementary nucleoside, and this postulation was also supported by the stability of the duplex with cDNA-3.

To elucidate the influence for the global structure of the modified duplex by the introduction of the cyclonucleoside, their CD spectra were observed at 20 °C (Figure 3). The duplex with both cDNA and cRNA found to form the right-handed helix, however, the overall shapes of both CD spectra were distinct from that of the unmodified duplexes. The positive Cotton band around 270 nm showed the drastic decrease and the blue shift. These results suggested that the introduction of the cyclonucleoside altered the duplex structure from typical structure to another one, and the blue shifts of the positive Cotton band indicated the modified duplex formed the loosened helical structure compared with the canonical duplex.

In conclusion, the novel oligonucleotide containing the cyclonucleoside, 6,3′-methanouridine derivative was prepared,

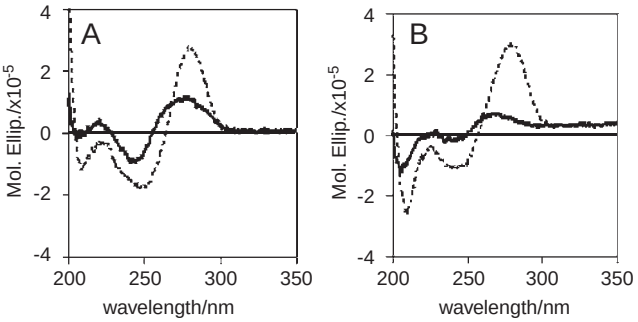

Figure 3. CD spectra of the duplex containing 6,3′-methanouridine derivative. (A) (—) ODN-1/cDNA, (- -) ODN-2/cDNA. (B) (—) ODN-1/cRNA, (- -) ODN-2/cRNA.

and the modified oligonucleotides formed the duplexes with the complementary DNA or RNA less stable than the unmodified ones. The helicity of the modified duplexes was loosened by the introduction of the cyclonucleoside. It seemed that the restricted torsion angle of the cyclonucleoside was distinct from that of the canonical nucleoside in both B-type and A-type duplexes.

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