Duplex Stabilizing Effect and Nuclease Resistant Property of Novel Oligonucleotides Containing C-2 Branched Polyamine-Bearing Deoxyinosine Derivative

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Novel oligodeoxyribonucleotides containing a C-2 branched polyamine-bearing deoxyinosine moiety at either inside-region or the 5′-terminus of the sequence exhibited enhanced nuclease resistant property. Among them, only the oligomers having the modified deoxyinosine residue at inside-region brought about duplex stabilizing effect upon mixing with the complements.

Using oligonucleotide for therapeutic and diagnostic purpose has been attracting a wide variety of interests of chemists as well as medical scientists and extensive study has been carried out to create a chemically modified DNA which is suitable for such use.[1,2] Modification of DNA through the introduction of cationic molecule such as polyamines seems to be attractive in order to increase the duplex stability consisting of modified DNA and the complementary oligonucleotide target.[3–10] Previously, we and others reported that the introduction of a branched-polyamine molecule at C-5 position of pyrimidine residue in a modified DNA, in such a way that the molecule lies in the major groove, resulted in increased duplex stability.[7,10] On the contrary, the introduction of a linear polyamine molecule at C-4 position of pyrimidine residue, in such a way that the molecule also lies in the major groove, decreased the duplex stability.[5] The same type of polyamine molecule, however, increased the stability when it was introduced at C-2 position of purine residue in the minor groove.[9] Thus, one has to consider about both the structure of the polyamine molecule and the location where the molecule would be positioned in order to achieve the enhanced duplex stability. These prompted us to synthesize novel modified DNAs which have C-2 branched polyamine-bearing purine derivative and study about the duplex stabilizing effect of the modified DNAs.

Two types of modified DNAs were synthesized. In GK-203 and -204, a normal deoxyguanosine residue at 4th position from the 3′-terminus is substituted with polyamine-attached deoxyinosine derivative. On the other hand, the same residue at the 5′-terminus is substituted with the inosine derivative in GK-205 and -206. These modified DNAs have either tris(2-aminoethyl)amine or tris(3-aminopropyl)amine as the polyamine function (Figure 1). The key intermediate, 5′-*O*-DMTr-2-fluoro-6-*O*-nitrophenylethyldeoxyinosine (**1**), and its phosphoramidite derivative (**2**) were prepared according to the procedure reported by Behr et al.[11] To check the feasibility of compound **1** as a precursor for the synthesis of modified DNAs, **1** was treated with tris(2-aminoethyl)amine/MeOH solution (1 : 1 v/v) at 60 °C for 24 h. After work up, deprotected polyamine-bearing nucleoside **3** was obtained in quantitative yield (Scheme 1).[12,13] Thus, the treatment of **1** with polyamine solution is effective both for the introduction of polyamine moiety and for the removal of nitrophenylethyl protecting group. Incorporation of the phosphoramidite **2** into oligoDNA was accomplished by an automated DNA synthesizer

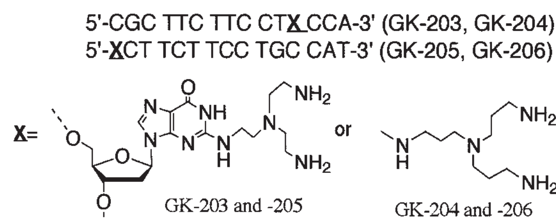
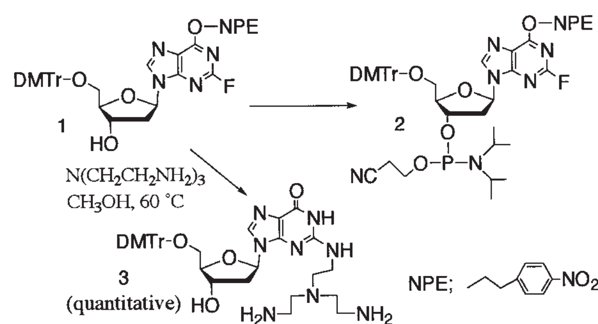


Figure 1. Structures of C-2 polyamine-bearing oligonucleotides.



Scheme 1. Reaction of protected 2-fluorodeoxyinosine.

(ABI 381-A). During the synthesis, prolonged coupling period (360 seconds) was applied for **2** otherwise the manufacture supplied standard procedure was followed. Under the condition, the coupling yield of **2** estimated by $DMTr^+$ assay[14] was higher (ca. 94%) than that of the previous report,[11] probably due to the prolonged coupling period. The obtained support-bound oligomer was treated with trisamine/MeOH solution as above. It should be noted that N^4-acetyl deoxycytidine (dC^{Ac}) phosphoramidite[15] instead of commonly used dC^{Bz} phosphoramidite was used in the synthesis to avoid the formation of possible N^4-transaminated products during the treatment. After work up, desirable modified DNAs (GK-203 to -206) were obtained in satisfactory yields.[16]

The obtained oligomers were digested by snake venom phosphodiesterase (SVPD, 3′-exonuclease), nuclease P1 (endonuclease) and alkaline phosphatase (AP) to confirm their structure. A typical HPLC analysis profile obtained for GK-203 is shown in Figure 2-a and the estimated nucleoside composition is listed in Table 1. As is clear from Table 1, the obtained nucleoside composition is parallel to the desired sequence. Interestingly, the same digestion of GK-203 without SVPD gave an unknown peak between the peaks of dT and dA while the peak corresponding to the polyamine-derived deoxyinosine (X) was missing as shown in Figure 2-b. The nucleoside composition analysis of the digest indicates that one dC residue and one X residue is missing. Further treatment of the digest with SVPD, however, gave a same HPLC profile as Figure 2-a (see Figure 2-c). These results indicate that the unknown peak in Figure 2-b is the dimer unit 5′-X(p)dC-3′. The results also suggest that the incorporation of C-2 polyamine-derived

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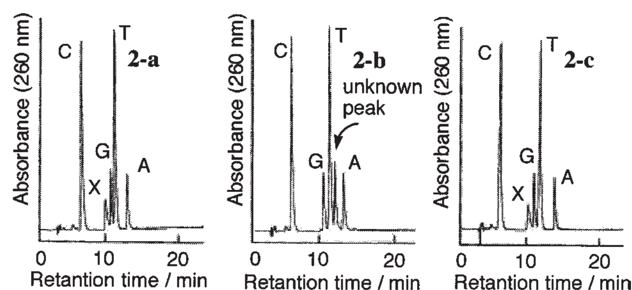

Figure 2. HPLC analses of the hydrolysate produced from the treatment of GK-203 using snake venom phosphodiesterase (SVPD), nuclease P1, and alkaline phosphatase (AP). (2-a) GK-203 treated with SVPD, nuclease P1, and AP. (2-b) GK-203 treated with nuclease P1 and AP. (2-c) Further treatment of the sample of 2-b with SVPD. The analyses were carried out by reversed phase HPLC on Wakoil 5C-18 column (4 mmϕ × 250 mm) using 0.1 M TEAA (pH 7.0) with a liner gradient (2.1–30.1%) of acetonitrile. X corresponds to C-2 tris(2-aminoethyl)amine-derived 2′-deoxyinosine.

Table 1. Results of nucleoside composition analysis[a]

	dA	dG	dC	T	X
GK-203	0.9(1)	1.2(1)	6.8(7)	5.2(5)	0.9(1)
GK-204	1.0(1)	1.0(1)	6.9(7)	5.2(5)	1.1(1)
GK-205	0.9(1)	0.9(1)	6.1(6)	6.0(6)	0.8(1)
GK-206	0.9(1)	1.0(1)	5.8(6)	6.1(6)	0.9(1)

[a]The number in parentheses indicate the sequence-based expected ratio of the nucleosides.

deoxyinosine into normal oligodeoxynucleotide suppress the susceptibility of the 3′-side phosphodiester bond of the modified nucleoside towards the action of the endonuclease. It should be noted that the incorporation of C-5 polyamine-derived 2′-deoxyuridine into normal oligodeoxynucleotide suppresses the susceptibility of the 5′-side phosphodiester bond of the modified nucleoside towards the action of SVPD[17] which is contrary to the current result.

The stability of the duplexes formed by the modified DNAs and their complements were analyzed by UV-melting experiments and Table 2 shows the obtained Tm values. cDNA-1 (15 mer), cDNA-2 (15 mer), and cDNA-3 (19 mer) were used as the complements. The Tm values of the unmodified oligomers (GK-203N and GK-205N) were also measured as the references. As is clear from Table 2, modified DNAs having the polyamine moiety at inside region (GK-203 and -204) bring about higher Tm values compared to that of GK-203N. Under the condition (pH 7.4), both of the primary amines in the polyamine molecule would exist as predominantly protonated form.[18] Thus, the observed Tm increment could be due to the interaction between the positively charged polyamine moiety and the negatively charged phosphodiester backbone. Meanwhile, the Tm values of GK-205 and -206 with complementary cDNA-2 were almost same as that of the corresponding unmodified oligomer (GK-205N), indicating the lack of such interaction. The increment of Tm values for both GK-205 and -206, however, was observed when the longer cDNA-3 was used as the complement (Table 2). Since cDNA-3 has extra nucleotide units in its 3′-region compared to cDNA-2, these results strongly suggest that the interaction does occur, at least in part, between the polyamine moiety and the phosphate backbone of the complements at the 3′ down-stream region of the complements from the counter position of the modified nucleoside. The possibility of specific interaction between the polyamine and the nucleobases in the minor groove like the case of spermine[19] is, however, not precluded.

The findings presented here will help designing functionalized DNA molecule possessing strong duplex stabilizing effect as well as the nuclease resistant property. Such features are both highly desirable in the case of using oligonucleotides as therapeutic and diagnostic tools.

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Table 2. Melting temperatures of the duplexes containing the modified oligomers

Duplex	GK-203N +cDNA-1	GK-203 +cDNA-1	GK-204 +cDNA-1	GK-205N +cDNA-2	GK-205 +cDNA-2	GK-206 +cDNA-2	GK-205N +cDNA-3	GK-205 +cDNA-3	GK-206 +cDNA-3
Tm/°C	57.1	60.6	59.8	59.1	58.7	58.3	59.3	61.9	61.8
ΔTm[a]	—	+3.5	+2.7	—	−0.4	−0.8	—	+2.6	+2.5

The UV-melting curves were measured at 260 nm with the oligomer concentration of 2 μM in sodium phosphate buffer (10 mM, pH 7.4) containing 100 mM of NaCl. The Tm values were obtained form first derivatives of the melting curves. The sequences of the complements are as follows; cDNA-1: 5′-TGG CAG GAA GAA GCG-3′, cDNA-2; 5′-ATG GCA GGA AGA AGC-3′, cDNA-3: 5′-ATG GCA GGA AGA AGC GGA G-3′. [a]The deviation from the Tm value of the corresponding duplex containing unmodified oligomer.