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Synthesis and enzymatic stability of oligonucleotides consisting of isonucleosides*

Zhen-Jun Yang, Hu-Yi Zhang, Ji-Mei Min, Ling-Tai Ma and Li-He Zhang†

School of Pharmaceutical Sciences, Beijing Medical University, Beijing 100083, China

Abstract: Novel oligodeoxynucleotide analogues consisting of deoxyisonucleosides were obtained by the phosphoramidite approach on automated DNA-synthesizer. The phosphoramidite building blocks were synthesized by phosphorylation of corresponding isonucleosides. The oligodeoxynucleotide analogues **A**, **B** and **C** were evaluated with respect to hybridization properties and enzymatic stabilities. The oligomers are all stable towards snake venom phosphodiesterase, but only oligomer **B** and **C** displayed acceptable hybridization properties to complementary dA_{14}.

One of the efforts to enhance the biological activity of oligonucleotides as inhibitors of gene expression has focused on the improvement of their stability to nuclease digestion. This problem has been approached in several ways, including alteration of the phosphate and sugar moieties in the oligonucleotides [1–3]. Most of the latter modifications contain a five-membered sugar ring closely resembling the natural deoxyribose [4–7]. Oligonucleotides incorporated with hexose nucleoside analogues were reported to have significant increase in stability towards phosphodiesterase and also retain hybridization properties [8]. Recently, an 'inverse oligonucleotides' was reported where the backbone of oligonucleotide consists of a phosphorylated cyclopentanediol moiety and the heterocyclic base is bound via a flexible ethylene linkage. But it was showed that the stability of duplexes with RNA and DNA is significantly reduced [9].

We are interested in the investigation of synthesis and enzymatic stability of oligodeoxynucleotides incorporated with isonucleosides. Isonucleoside is a new class of nucleoside analogues in which the nucleobase is linked to the position of ribose other than C-1′. Therefore, two trideoxynucleotides incorporated with 3′-(S)-(thymin-1-yl)-4′-(R)-hydroxy-5′-(S)-hydroxymethyl tetrahydrofuran [10] 1 in the 3′ end 2 or in the center 3 were synthesized via phosphotriester method in solution. Compounds 2 and 3 are stable towards Nuclease S1 compared with the normal trideoxynucleotide 4 [11]. The torsion angles in the sugar phosphate backbones of the three trimers were calculated by using 'Amber Program' in SGI IRIS XS24. The great changes were found in compounds 2 and 3, these alterations in torsion angles might affect the recognition of nuclease to substrate. These characters prompt us to research on the hybridization properties of oligodeoxynucleotides incorporated with other isonucleosides.

Compound 1 ($[\alpha]_D^{20}+17.2$, c 0.702, MeOH) was synthesized from D-xylose and its enantiomer 3′-(R)-(thymin-1-yl)-4′-(S)-hydroxy-5′-(R)-hydroxymethyl tetrahydrofuran 5 ($[\alpha]_D^{20}-16.7$, c 0.647, MeOH) was obtained from L-xylose [12]. The corresponding phosphoramidite were synthesized as building blocks by standard procedure. Oligodeoxynucleotides A and B were prepared on Applied Biosystems 381A DNA synthesizer using a standard Applied Biosystem cycle (0.2 μmol scale). The composition of the oligomer A and B were verified by matrix assisted laser desorption mass spectrometry which gave a relative molecular mass of 4215.5 ($M^+ + Na^+ - H^+$) and 4194.4 (M^+) (calc. 4194.7), respectively. The substrate activities of the novel oligodeoxynucleotide analogues A and B were tested against snake

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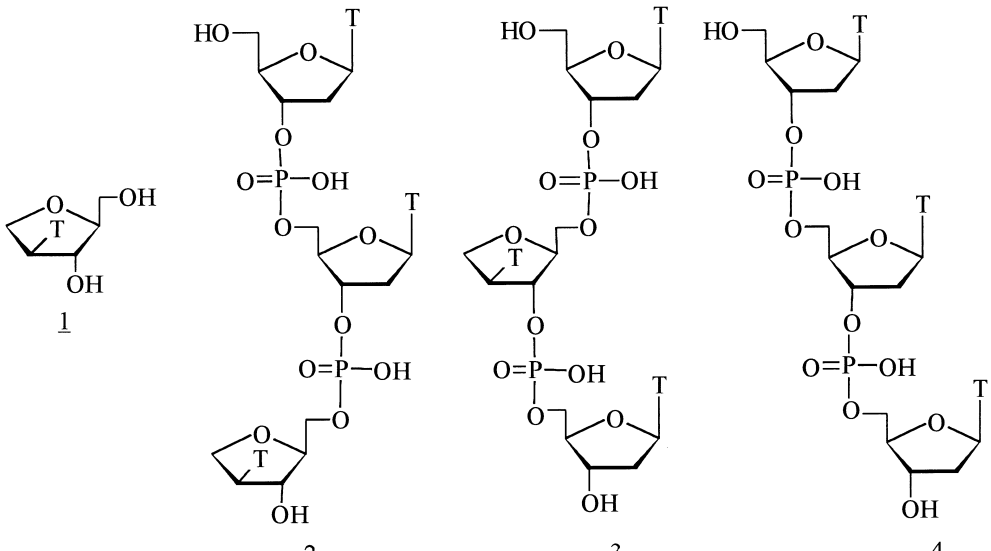
†*Corresponding author.*

venom phosphodiesterase (SVPDE). The result indicated that no significant change of UV absorbance occured within 30 mins when the oligomers A or B were incubated with snake venom phosphodiesterase (Buffer, 0.1 M Tris-HCl; pH 8.6; 0.1 M NaCl; 14 mM $MgCl_2$. Conc. of each oligonucleotide, 1 μM. Conc. of SVPDE, 2.4×10^{-3} units/mL), while dT_{14} gave way as expected to a time dependant increase of absorbance. The hybridization properties of the two novel oligodeoxynucleotide analogues A and B towards their complementary strand dA_{14} were determined by melting temperature (T_m) measurements with a SWIFT-Tm Software on the UV spectrophotometer. The results are given in Table 1. It was showed that the oligomer B consisting of isonucleoside 5 could form a duplex with the complementary oligodeoxynucleotide although a slight depression of T_m was found. But no typical T_m curve was observed in the case of the oligomer A containing of isonucleoside 1. By means of computer modeling, the total energy of the duplex formed by oligomer B with dA_{14} is lower than that from oligomer A ($\Delta E = 56.9$ kCal) (Scheme 1).

Table 1 Melting temperatures of oligodeoxynucleotides incorporated with isonucleosides

Sequence and duplex	T_m (°C)	ΔT_m (°C)
dA_{14}/dT_{14}	38.2	–
dA_{14}/**A**	< 20	–
dA_{14}/**B**	32.3	5.9
dA_{14}/**C**	33.5	4.7
$dA_{14}/(\mathbf{1})_1{\cdot}dT_{13}$ (**D**)	36.3	1.9
$dA_{14}/dT_7{\cdot}(\mathbf{1})_1{\cdot}dT_6$ (**E**)	24.0	14.2
$dA_{14}/(\mathbf{6})_1{\cdot}dT_{13}$ (**F**)	36.9	1.3
$dA_{14}/dT_7{\cdot}(\mathbf{6})_1{\cdot}dT_6$ (**G**)	24.8	13.4

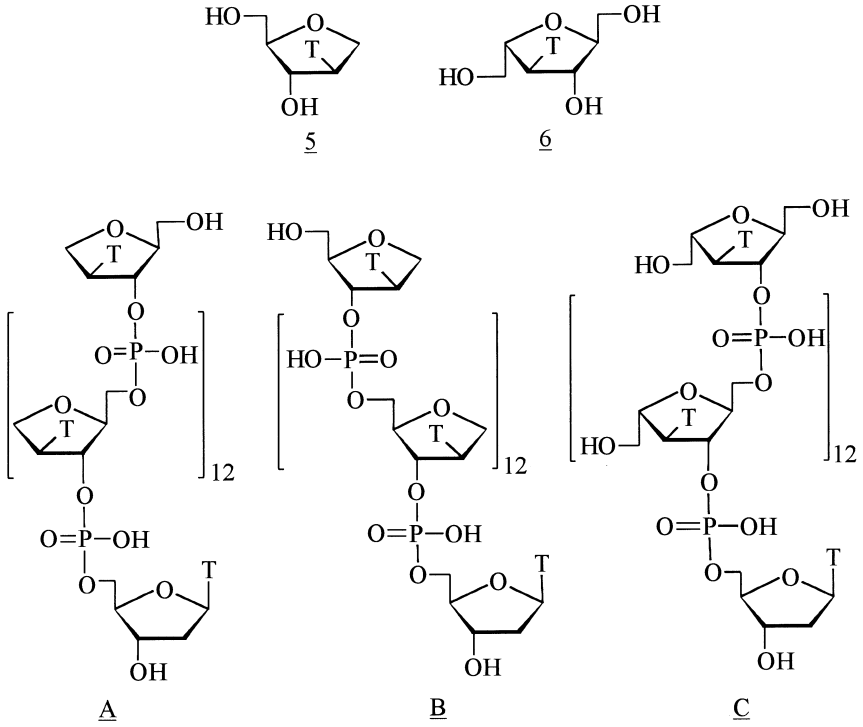
T_m, Melting temperature of the oligonucleotides annealed to dA_{14} determined under the following condition: Buffer, 0.14 M NaCl, 0.01 M Na_2HPO_4, pH 7.2, 1.0 mM EDTA. Conc. of each oligonucleotide, 2 μM. ΔT_m, Decrease in T_m compared with the T_m of normal duplex.



Scheme 1

Recently, a number of nucleosides with the unnatural L-configuration have been reported as potent chemotherapeutic agents against HIV, HBV and certain forms of cancer. It is interesting that these L-nucleosides have potent biological activities, while some of them show lower toxicity profiles than their D-counterparts [13]. We have reported the synthesis of 4-deoxy-4-nucleobase-2,5-anhydro-L-mannitol derivatives [14]. These structures are very similar to isonucleoside 1 except an additional hydroxymethyl

group at the 5′ position. In order to gain a better understanding of the stereochemistry that would be required for the formation of duplex with oligonucleotides incorporating isonucleosides, we investigated the synthesis, enzymatic stability and hybridization properties of oligonucleotide incorporating with 4-deoxy-4-(thymin-1-yl)-2,5-anhydro-L-mannitol 6. Oligomer C and its analogues F, G were obtained on DNA synthesizer. In order to compare the structural stability of the modified oligonucleotide, oligomers D and E were also synthesized. In Table 1, it is showed that all of the oligomers B, C, D, E, F, G can form a stable 1:1 complex with dA_{14}. Although an incorporation of isonucleoside has a negative effect on the T_m value of the duplex, the oligomers incorporated with isonucleoside 6 (C, F, G) give a higher T_m value than the corresponding oligomers B, D, E. It means that introduction of hydroxymethyl group in compound 1 makes structural stability prevailing the distortions caused by transposition of nucleobase at the sugar ring. Compound 1 and 5 are enantiomers of deoxyribonucleoside, the structure of compound 6 is similar to ribonucleoside. It is well known that RNA:DNA heteroduplexes appear to be more stable to thermal denaturation than their DNA:DNA counterparts. Therefore, the greater stability of dA_{14}:C heteroduplex over dA_{14}:A and dA_{14}:B homoduplexes seems to be dominated by an additional hydrogen bonding caused by hydroxymethyl group of compound 6. Enzymatic hydrolysis of oligomer A, B, C was studied and showed significant resistance of the phosphodiester bonds towards snake venom phosphodiesterase (Scheme 2).



Scheme 2 D 5′-$(5)_1$•$(dT)_{13}$-3′; E 5′-$(dT)_7$•$(5)_1$•$(dT)_6$-3′; F 5′-$(6)_1$•$(dT)_{13}$-3′; G 5′-$(dT)_7$•$(6)_1$•$(dT)_6$-3′. T = Thyminyl.

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