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Synthesis and Structural and Thermodynamic Properties of Oligonucleotides Containing 2'-*O*-Phosphorylated Ribonucleosides

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Abstract: Conformation of the sugar, backbone and glycosidic bond of synthetic 2'-*O*-phosphorylated diuridylate U(2'-p)pU **1** was studied by NMR and CD. It was found that the 5' upstream ribose has predominantly an S-type of conformation. Tridecadeoxyuridylates incorporating a 2'-*O*-phosphorylated uridine U(2'-p) at the 3rd or 7th position were prepared by use of the polymer support synthesis and the effect of the 2'-*O*-phosphoryl group on the stability of the DNA duplex with dA_{13} was examined. Both Tm experiments of these DNA duplexes and MD simulations of a duplex of $dU_3U(2'\text{-p})dU_3/dA_7$ suggested U(2'-p) has an extremely rigid *S*-type conformation when incorporated into the DNA duplex.

In splicing of certain pre-tRNAs having an intron, tRNAs containing a phosphoryl group at the 2'-position of a nucleoside near the anticodon 3'-side have been found as initial products.[1] Szostak *et al.* have eventually found the 2'-*O*-thiophosphorylated RNA species during their studies of *in vitro* selection of RNA ribozymes bearing a 5'-thiophosphorylation activity.[2] Interestingly, they also revealed that reverse-transcription of an RNA oligomer having a 2'-*O*-thiophosphoryl group as the template caused a pause of AMV or MMLV H$^-$ reversetrascriptase, which ultimately read through this point.[3] Therefore, studies on the 3D-structure of 2'-*O*-phosphorylated RNAs would provide valuable information for molecular recognition of ARSs and HIV-reversetranscriptases. Quite recently, we have reported the synthesis of oligouridylates (2-6mers) phosphorylated at all internal 2'-positions by use of a phosphoramidite unit **6**.[4] This paper describes the conformational analysis of U(2'-p)pU **1**, incorporation of 2'-*O*-phosphorylated uridine U(2'-p) into oligodeoxyuridylates by an improved phosphoramidite approach on a controlled pore glass (CPG), and thermodynamic properties of the DNA duplexes of these oligomers with complementary oligodeoxyadenylates.

As shown in Figure 1, the CD spectra of **1** exhibited only a little change in the θ value *versus* wavelength profiles at 0, 20 and 60 °C. It is, however, noted that the CD profiles of **1** were quite different from those of diuridylate UpU **2** but very close to those of d(UpU) **3** as far as the whole shape of the spectra and intensity of the negative and positive Cotton effects at nearby 240 and 270 nm, respectively, are concerned. These results suggested that U(2'-p)pU **1** might have a B-DNA type of conformation in an aqueous solution.

All the ^3J values of the sugar protons in **1** were determined by NMR experiments to study the detailed analysis of conformation of the 5'-upstream ribose.[5] In general, the ^3J values obtained by ^{1}H, ^{13}C and ^{31}P NMR provide information on the ribose pucker and torsion angles of the backbone structure. In an aqueous solution, the sugar pucker of nucleosides and nucleotides is known to exist in equilibrium between the two interconvertable conformers denoted as S-type (C2'-endo) and N-type (C3'-endo),[6] which are seen in canonical B-DNA and A-RNA, respectively. The ratio of the two conformers can be estimated by the Altona's equation, *i.e.*, $S\% = (J_{1',2'}-1) / 6.9 \times 100$.[7] According to this calculation, the ribose having the 2'-*O*-phosphoryl group was markedly oriented to the S-conformer to a high degree of 81%. Another equation, $S\% = J_{1',2'} / (J_{1',2'}+J_{3',4'}) \times 100$,[8] also gave a similar result (S% = 73%). These results were in good agreement

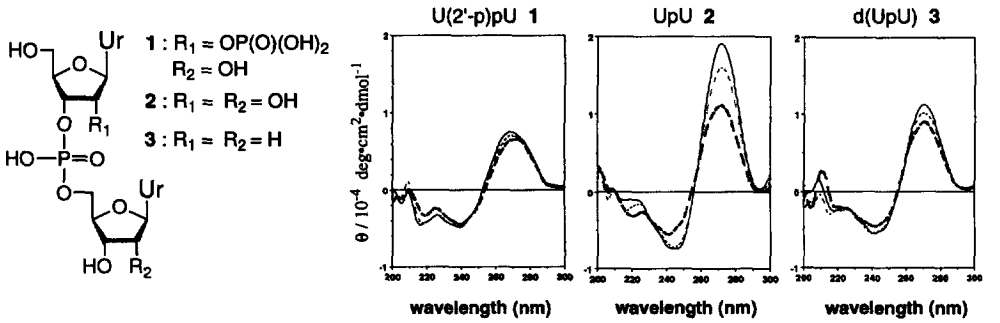


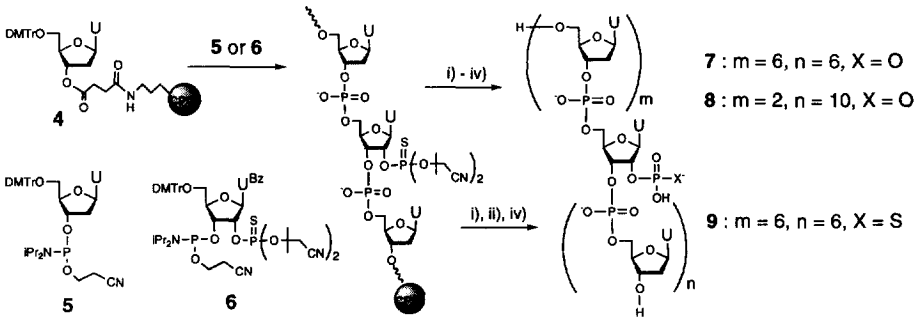
Figure 1. The CD spectra of **1**, **2** and **3** in 0.1 M NH_4OAc (pH 7.0) at 0 (—), 20 (- - -) and 60 (- - -) °C.

with that observed in the CD spectra of **1**, *viz.*, preference of a C2'-endo conformation similar to that of B-DNA. To our best knowledge, a few examples have been reported of ribonucleotides having such a rigid S-type sugar pucker.[9] Based on calculation using the $^3J_{POCC}$ values of C4'-P3' and C2'-P3' (2.0 and 4.6 Hz, respectively),[10] the C3'-O3' torsion angle ε was determined as *gauche*$^-$. This backbone conformation is one of the characteristic features of **1** since mono- or oligo-nucleotides usually have the *trans*-conformation. This property is undoubtedly ascribed to electrostatic repulsion between the negative charges of the two proximal phosphoryl groups. According to the NOESY spectrum of **1**, no interresidual nOe cross peaks could be observed, while intraresidual nOe between H6 and H2' in the 5'-upstream U was clearly observed so that the glycosidic conformation could be determined as *anti*. Our preliminary results of NMR and CD studies using other 2'-*O*-phosphorylated dimers, N(2'-p)pN (N = U or A), also suggested similar conclusions. Therefore, this conformational property is independent of the kind of base moiety and is inherent in 2'-*O*-phosphorylated dimers.

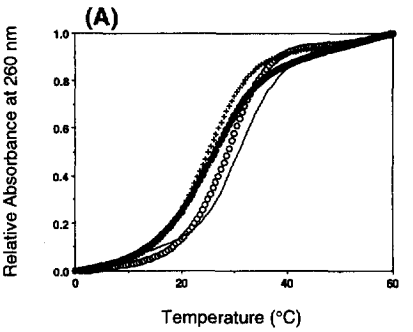
Next, on the basis of these unusual structural properties of **1**, incorporation of a 2'-*O*-phosphorylated ribonucleoside U(2'-p) into DNA oligomers was examined by use of the polymer support synthesis. The partially 2'-*O*-(thio)phosphorylated oligodeoxyuridylate 13mers **7**, **8** and **9** were synthesized on deoxyuridine-loaded aminopropyl-CPG **4** using the uridine phosphoramidites **5** and **6** which were prepared as described in our previous paper.[4] In the case of condensation using **6**, the reaction was performed at a slightly high concentration (0.15 M) of the phosphoramidite reagent. In the synthesis of **7** and **8**, after the chain elongation, the 2-cyano-1,1-dimethylethyl (CME) and 2-cyanoethyl (CE) groups were removed from the fully protected oligomer on the polymer support by use of DBU in the presence of *N,O*-bis(trimethylsilyl)acetamide (BSA)[11] without release of the oligomers from the CPG. Conversion of the 2'-thiophosphate group to a 2'-phosphate group was successively carried out by treatment with KI_3,[12] which was more effective than iodine[13] in this reaction. If iodine was used instead of KI_3 in the oxidative S-O conversion, 5-iodination of the unprotected uridine residue was observed to an appreciable extent. Finally, the succinate linker was cleaved by treatment with conc. NH_3 and the products were purified by HPLC to give **7** and **8** in 28% and 16% yields, respectively. The oligomers **7** and **8** were characterized by enzymatic treatment with alkaline phosphatase, which gave the 2'-dephosphorylated products dU_6UdU_6 and dU_2UdU_{10}, respectively. The oligomer **9** was also synthesized by a modified procedure without the KI_3 treatment and characterized by the facile 2'-dethiophosphorylation using thermolysis at pH 7 and 90°C for 20 min, which was recently reported by us,[14] to give dU_6UdU_6 in nearly quantitative yield without appreciable internucleotidic bond cleavage.

Furthermore, the UV-melting curves for duplexes between these oligomers and complementary dA_{13} were measured to study the effect of the 2'-*O*-phosphoryl group on the duplex stability. Compared with the Tm value of unmodified dU_{13} **10**, those of **7** and **8** (26.2 and 28.8 °C, respectively) were only slightly lower than expected, despite the disadvantageous electrostatic repulsion due to the 2'-phosphomonoester. This was

probably because the 2'-*O*-phosphorylated uridine incorporated has a rigid 2'-endo conformation favorable for the DNA duplex. On the other hand, the duplex of **9** was destabilized more significantly by 5.4 °C than that of **7**. This might be explained by the more unfavorable steric disruption due to the sulfur atom as well as stronger electrostatic repulsion due to the 2'-thiophosphate dianion species. It is known that the pKa values of monoalkyl esters of thiophosphoric acids are generally 0.5 unit less than those of the corresponding monoalkyl esters of phosphoric acids[15] so that the electrostatic charge distribution of the 2'-thiophosphate group is greater than the 2'-phosphate at pH 7.0.



Scheme 1. Synthesis of partially 2'-phosphorylated oligodeoxyribonucleotides i) 0.2 M DBU / BSA-pyridine (1:1, v/v), 2 h. ii) pyridine-H_2O (9:1, v/v). iii) 0.1 M KI_3 / pyridine-H_2O (9:1, v/v), 24 h. iv) conc. NH_3-pyridine (9:1, v/v), 15 h



(B)

	Oligomer	Tm (°C)	ΔTm (°C)
——	dU_{13} **10**	30.9	
●●●	$dU_6U^{2'PO}dU_6$ **7**	26.2	-4.7
○○○	$dU_2U^{2'PO}dU_{10}$ **8**	28.8	-2.1
+++	$dU_6U^{2'PS}dU_6$ **9**	25.5	-5.4

Figure 2. UV-melting curves (A) and Tm values (B) for duplex of partially 2'-phosphorylated oligodeoxyuridylate 13mers (**6**-**9**) and dA_{13} in 10 mM Na_2HPO_4, 1 M NaCl, 0.1 mM EDTA (pH 7.0), 2.0 μM oligonucleotide

To study the structural effects of the 2'-*O*-phosphoryl group on the duplex, computational molecular dynamics (MD) simulations were carried out by using the duplexes of oligodeoxyuridylate 7mers (**11**: dU_7 and **12**:$d(U)_3U(2'\text{-}p)d(U)_3$) and dA_7 for 1 nsec in vacuum.[16] Figure 3 shows the MD trajectory of phase angle *P* of the ribose in the 4th uridine residue whether phosphorylated at the 2'-position or not. As shown in Figure 3A, the puckering of the unmodified 4th uridine in **11**/dA_7 duplex was relatively flexible and frequently changed from S-type to N-type conformers ($-1° < P < 34°$), and *vice versa*. On the contrary, the 2'-*O*-phosphorylated sugar in **12**/dA_7 moved sluggishly only within the range of S-type conformers ($137° < P < 194°$) during the 1 nsec-MD simulation. This result strongly suggests that the ribose residue of the 2'-*O*-phosphorylated uridine incorporated into the DNA duplex exists in 2'-endo conformation rigidly, as discussed above.

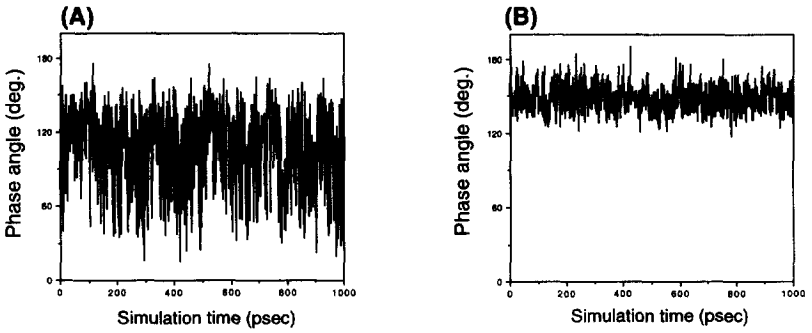


Figure 3. Variation of the phase angle of the 4th deoxyuridine (Panel A) and 2'-phosphorylated uridine (Panel B) residues in duplexes of dU_7/dA_7 and $dU_3U(2'\text{-p})dU_3/dA_7$, respectively, in the MD simulations for 1 nsec.

The present study disclosed the unique structure and MD behavior of oligonucleotides containing a 2'-*O*-phosphorylated uridine. Further studies on the synthesis and properties of RNA oligomers containing 2'-*O*-phosphorylated ribonucleosides, such as a partial anticodon-stem and loop structure of tRNA splicing products, are now in progress.

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