INHIBITION OF RESTRICTION ENDONUCLEASE CLEAVAGE BY TRIPLEX FORMATION WITH OLIGO-2'-*O*-METHYL-RIBONUCLEOTIDES CONTAINING 8-OXO-2'-*O*-METHYLADENOSINE IN PLACE OF CYTIDINE[†]

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Abstract - The ability of homopyrimidine oligoribonucleotides and oligo-2'-*O*-methyl-ribonucleotides containing 8-oxo-adenosine (AOH) and 8-oxo-2'-*O*-methyl-(AmOH) adenosine to form stable, triple-helical structures with sequences containing the recognition site for the class II-S restriction enzyme, *Ksp*632-I, was studied as a function of pH. The AOH- and AmOH-substituted oligoribonucleotides and oligo-2'-*O*-methyl-ribonucleotides were shown to bind within the physiological pH range in a pH-independent fashion, without a compromise in specificity. The substitutions of three cytidine residues with AOH showed higher endonuclease inhibition than the substitution of either one or two cytidine residues with AOH. In particular, the oligo-2'-*O*-methyl-ribonucleotide with only one cytidine substituted with AmOH showed higher endonuclease inhibition. Increased resistance to nucleases is observed with the introduction of 2'-*O*-methylnucleosides. This stabilization should help us to design much more efficient third strand homopyrimidine oligomer and antisense nucleic acid, which can be used as tools in cellular biology and anti-viral therapy.

INTRODUCTION

Homopyrimidine-homopurine sequences have been mapped to several sites in the regulatory regions of eukaryotic genes, and are hypersensitive to single-strand-specific nucleases, such as S1.[1-3] These sequences are expected to exhibit unusual DNA structures, as they are known to undergo a transition to an underwound state in plasmids, under conditions of moderately acidic pH and negative supercoiling. Studies of these systems appear to support a model consisting of a triple-strand (pyr-pur-pyr) plus a single-stranded structure namely H-DNA.[4-8] Pyrimidine oligonucleotides bind in the major groove of the DNA, parallel to the purine Watson-Crick strand, through the formation of specific Hoogsteen hydrogen bonds to the purine Watson-Crick bases. The specificity arises from Hoogsteen hydrogen bonding, in which a thymine (T) in the third strand recognizes adenine-thymine (AT) base pairs in the double-stranded DNA (T-AT triplet), and a protonated cytosine (C+) in the third strand recognizes guanine-cytosine (GC) base pairs (C+GC triplex).[9-14] However, protonation at the N-3 of C is required in order to form two hydrogen bonds with the G of the target strand. Due to this requirement, triplex formation by homopyrimidines that contain multiple C residues is sensitive to pH and is usually restricted to pH values of 7 or below.

Recently, the triple helix forming abilities of N^6-methyl-8-oxo-2'-deoxyadenosine,[15,16] 8-oxo-2'-deoxy-

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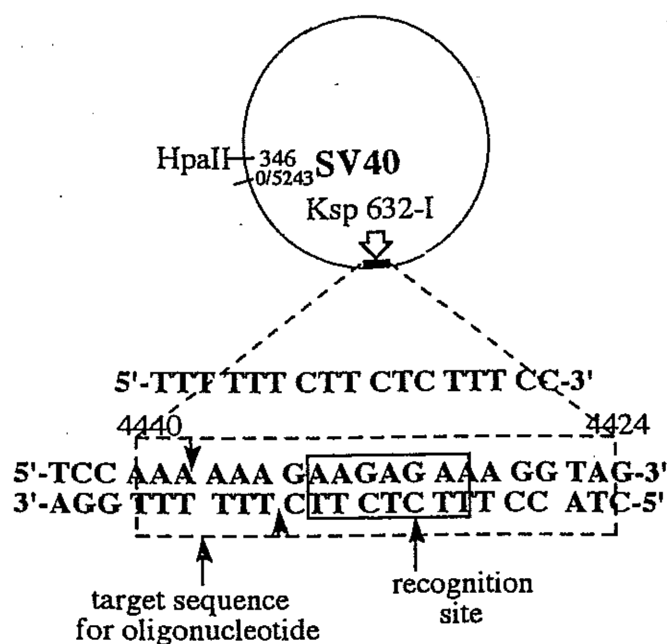
[†]Dedicated to Professor Terrace Mukaiyama on the occasion of his 73rd birthday.

adenosine,[17,18] and pseudoisocytidine[19] as protonated deoxycytidine analogues were shown to be pH-independent within the physiological range. Furthermore, Shimizu *et al.*[20] have shown that the triplexes formed with oligo-(2'-methoxylribonucleotides) or (2'-fluororibonucleotides) were more thermally stable than those formed by DNA oligonucleotides. The 2'-modified RNAs may bind more effectively and tightly to the DNA duplex than a third DNA strand, if the 2'-modified RNAs have the C3'-*endo* conformation and the 2'-substituted groups enhance the rigidity of the triple-helix structure. However, the oligo-2'-*O*-methyl-ribonucleotides bind in a pH-dependent manner to the target duplex.



	u-ODN	5'AAAAAAGAAGAGAAAGG3'
	y-ODN	5'TTTTTTCTTCTCTTTCC3'
1		5'UUUUUUCUUCUCUUUCC3'
2		5'UUUUUUCUUAOHUCUUUCC3'
3		5'UUUUUUAOHUUCUAOHUUUCC3'
4		5'UUUUUUAOHUUAOHUAOHUUUCCC3'
5		5'UmUmUmUmUmUmCmUmUmCmUmCmUmUmUmCmCm3'
6		5' UmUmUmUmUmUmCmUmUmAmOHUmCmUmUmUmCmCm 3'
7		5' UmUmUmUmUmUmAmOHUmUmCmUmAmOHUmUmUmCmCm 3'
8		5' UmUmUmUmUmUmAmOHUmUmAmOHUmAmOHUmUmUmCmCm 3'

u-ODN:homopurine oligonucleotide; y-ODN:homopyrimidine oligonucleotide.

Figure 1. Schematic representation of SV40 DNA, showing the recognition site for the *Ksp* 632-I enzyme and the target sequences for the homopyrimidine oligonucleotides containing 8-oxo-adenosine and 8-oxo-2'-*O*-methyladenosine, which are shown above the boxed target sequence. *Ksp* 623-I and *Hpa* II were used for SV40 DNA linearization. Short arrows indicate the cleavage sites for the restriction endonucleases.

In this paper, we wish to report the possibility of inhibiting sequence-specific DNA binding proteins by oligoribonucleotides and oligo-2'-*O*-methyl-ribonucleotides containing 8-oxo-2'-adenosine (AOH) and/or 2'-*O*-methyladenosine (AmOH) instead of cytidine (Figure 1). We have tested the ability of homopyrimidine oligoribonucleotides and oligo-2'-*O*-methyl-ribonucleotides containing AOH and/or AmOH to inhibit the sequence-specific cleavage of simian virus 40 (SV40) DNA, at neutral and basic pH values, by the class II-S restriction endonuclease, *Ksp* 632-I.[21,22]

MATERIALS AND METHODS

Oligonucleotide synthesis. 5'-*O*-(4,4'-Dimethoxytrityl)-8-hydroxyl-2'-*O*-(*tert*-butyldimethylsilyl)- and 8-hydroxyl-2'-*O*-(methyl)-N^6-acetyladenosine-3'-*O*-(2-cyanoethyl*N,N*-diisopropylphosphoramidites) were synthesized by the procedures of Kim *et al.*[23] Oligonucleotides were synthesized on an Applied Biosystems Model 392 DNA/RNA automatic synthesizer on a 1 μM scale with ribonucleotide phosphoramidite reagents; standard phosphoramidite chemistry was employed. The oligoribonucleotides were base-deprotected by an incubation in 3 mL of aqueous concentrated ammonia (28%)/ethanol (3:1) for 16 h at 55℃ in a screw-cap glass vial. After complete removal of the solvent by evaporation, the residue was taken up in 0.5 mL of 1.1 M tetrabutylammonium fluoride in THF and was left at room temperature overnight. An OPC reverse phase cartridge (PerSeptive Biosystems) was used for simple purification after desilylation of the RNA. The cartridge was first washed with 4% acetonitrile in 100 mM triethylammonium bicarbonate (pH 7.5) to remove the impurities. The RNA was then eluted with acetonitrile-methanol-water (3:3:4) and was evaporated *in vacuo*. The crude RNA was purified by chromatography on a YMC column with a linear gradient of CH_3CN in 0.1M triethylammonium acetate (pH7.0). After purification, the fragments were lyophilized to dryness. Circular SV 40 DNA was linearized with the restriction enzyme *Hpa* II, which cleaves at position 346. The cleavage products were separated on a 0.8% agarose gel. The products were eluted from the gel, and were recovered from the eluate by ethanol precipitation.

Thermal denaturation profiles. Thermal transitions were recorded at 260 nm using a Shimadzu UV-2200 spectrophotometer. The insulated cell compartment was warmed from 5°C to 90°C, with increments of 1°C and equilibration for 1 min after the attainment of each temperature, using a temperature controller, SPR-8 (Shimadzu). Samples were heated in masked 1 cm path length quartz cuvettes fitted with Teflon stoppers. Each thermal denaturation was performed in 33 mM Tris/acetate buffer (pH 6.0, 7.0, and 8.0), 1mM EDTA, 10 mM $MgCl_2$, and 100mM NaCl, containing 1 μM of each strand. The mixture of duplex and single strands was kept at 90℃ for 5 min, and then was cooled to 5℃. At temperatures below 20℃, N_2 gas was continuously passed through the sample compartment to prevent the formation of condensate.

Gel electrophoresis. The duplex DNA was made by combining the designated concentrations of oligonucleotides in 33 mM Tris-acetate (pH 7.0) buffer/100 mM NaCl/10 mM $MgCl_2$, incubating the mixture at 90°C for 10 min, and slowly cooling it to room temperature. The triplex oligonucleotides were made by the addition of an equimolar amount of the third strand to the duplex, followed by an incubation at 4°C overnight. The concentration of each strand was 1.23 X 10^{-3} μM in a total vol. of 10 μL. Electrophoresis experiments were conducted using gels containing 15% polyacrylamide (acrylamide/bisacrylamide, 19:1) prepared in a Bio-Rad Protean II gel apparatus with 20 X 22 X 0.75 cm glass plates. Tris/borate buffer (50 mM, pH 7.8)/5

mM $MgCl_2$ was used in the electrophoresis reservoirs. Electrophoresis was conducted at a constant temperature (4°C) and 200V for 16h. The gel was stained using methylene blue.

Exonuclease stability of oligonucleotides. The oligonucleotide was labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The oligonucleotides (0.1 μM) were incubated with 100 μL of culture medium containing 10% fetal bovine serum for 24 h at 37 °C. Aliquots (5μL) of the reaction were removed at the indicated times (15, 50, 60, 120, 240, 480, 960, and 1440 min), added to an equal volume of 80% deionized formamide containing 0.1% xylene cyanol and 0.1% bromophenol blue, and analyzed by electrophoresis on 20% polyacrylamide/7 M urea (29:1) gels. Autoradiograms were obtained by exposing the gels to Fuji (X-ray) film at –20°C. The extent of cleavage was determined by comparing the radioactivity of the intact fragment to that of the cleaved fragments with a BioImage analyzer, BAS 2000 (Fuji Medical Systems USA)

Inhibition of restriction endonuclease digestion at *Ksp* 632I sites in SV40 linear DNA. Enzymatic assays were performed in a buffer containing 33 mM Tris-acetate, 100 mM NaCl, 66 mM potassium acetate (pH 7.0 and 8.0), 0.5 mM dithiothreitol, 10 mM $MgCl_2$, 1 mM spermine, and 1μg/μL tRNA at 30°C.[24] The pH of the incubation medium was changed as indicated in the text and the legend of the figure. The concentration of SV40 linear DNA was usually 6 nM, and 20 units/μL restriction enzyme were used in each assay. After the incubation, the enzymatic reactions were stopped by the addition of EDTA (10 mM). Specimens were then analyzed by electrophoresis on slab gels (0.8% agarose). Densitometric analysis of gels stained with ethidium bromide was performed on a Millipore Bio Image 60S densitometer.

RESULTS AND DISCUSSION

In order to investigate whether imperfect recognition sites, containing more than a C-G inversion, can form triplex structures, we synthesized the 17 nucleotide duplex, 5'AAAAAAGAAGAGAAAGG3'/ 3'TTT-TTTCTTCTCTTTCC5' (SV40 target sequence), and studied its interactions with the homo-pyrimidine RNA and 2'-*O*-methyl RNA oligonucleotide analogues containing AOH and/or AmOH instead of cytidine (Figure 1). The ability of the oligonucleotides to form triple helices was examined by the melting temperature studies. Table 1 shows the results of the thermal melting of the homopyrimidine oligoribonucleotides and oligo-2'-*O*-methyl-ribonucleotides containing AOH and AmOH (**1**-**8**), bound to the 17 mer duplex. The influence of

Table 1. Melting Temperatures, Tm (°C), of the Triplexes[a] from with the Modified Oligonucleotides (**1**-**8**)

Oligonucleotide Number	Tm (°C)		
	pH6.0	pH7.0	pH8.0
1	46	29	25
2	35	32	26
3	30	31	24
4	28	28	27
5	52	30	28
6	34	34	34
7	37	36	36
8	40	39	38

a) Duplex, 5'AAAAAAGAAGAGAAAGG3'/3'-TTTTTTCTTCTCTTTCC5'

the pH on the Tm was determined at pH 6.0, 7.0, and 8.0 for the 17 mer duplex/unmodified and modified homopyrimidine oligoribonucleotides (**1-8**). The unmodified, **1**, and modified (AOH; **2** and **3**) homopyrimidine oligoribonucleotides bound in a pH-dependent manner to the target duplex. Thus, an increase in the pH should not favor the protonation of cytidine, and thereby should decrease the stability of the triplex. In contrast, the oligonucleotide with the complete substitution of AOH for the cytidine base residues (**4**) exhibited pH-independent binding in the physiological range (pH 6.0-8.0). On the other hand, the oligo-2'-*O*-methyl-ribonucleotides with from one to three substitutions of AmOH showed pH-independent binding in the physiological range. Of particular interest was the oligo-2'-*O*-methyl-ribonucleotide with only one substitution of AmOH, which was found to have pH-independent binding of the same order as that for the oligomer (**4**). In a previous study, we tested the pH-independent binding for homopyrimidine oligodeoxyribonucleotides containing 2'-modified nucleosides (Um, Cm, Uf, and Cf), but the results showed the same pH dependence as the pyrimidine-rich DNA oligomers.[25] The mixed oligonucleotides containing 2'-modified nucleosides and 8-oxo-2'-deoxyadenosine (dAOH) destabilized the triplex by the incorporation of dAOH at pH 6.0-8.0. This triplex destabilization is influenced by the different conformation of the dAOH (*syn*) and the 2'-modified nucleosides (*3'-endo*). However, the oligo-2'-*O*-methyl-ribonucleotides enhance the Hoogsteen bond stability by the incorporation of the AmOH at pH 6.0-8.0. These results suggest that the conformation of the 3'-endo sugar of the third strand pyrimidine and the *syn* conformation of the adenosine base favor the triple-helix structure and the increased pH independence of the binding of the triplex, due to the 2'-*O*-methyl and 8-hydroxyl groups. Since 8-oxo-2'-deoxyadenosine contains two hydrogen bond donors, at positions N-7 and N-6, and the pK of the hydrogen at position 7 is approximately 8.7, its ability to participate in triplex formation should not be affected by an increase in the pH. 8-Oxo-2'-deoxyadenosine has been shown to exist in the keto form, with the base in the *syn* conformation,[26,27] and recent NMR results suggested that when incorporated in a DNA duplex, 8-oxo-2'-deoxyadenosine exists in the keto form, although in this case, the base appears to adopt an *anti* conformation in the duplex.[6] The formation of a similar hydrogen bond was recently proposed by Young *et al.*[15] for the interaction of N^6-methyl-8-oxo-2'-deoxyadenosine with GC base pairs in duplex DNA.

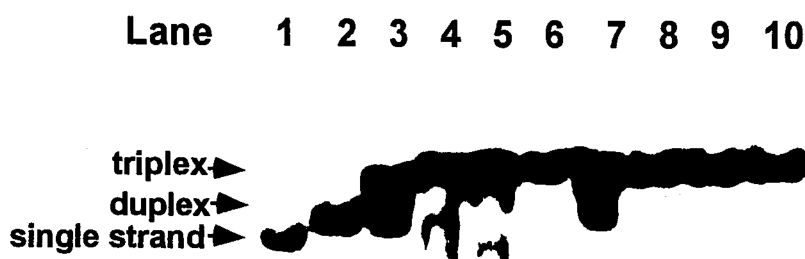


Figure 2 . Methylene-blue stained, 15% non-denaturing polyacrylamide gel run in 33 mM Tris/acetate buffer (pH 7.0), 100 mM NaCl, and 10 mM $MgCl_2$. Lane 1:ODNs; lane 2:duplex DNA; lanes 3-16:oligomers (**1-8**).

Next, the formation and the stability of triplexes with oligoribonucleotides and oligo-2'-*O*-methyl-ribonucleotide analogues containing 8-oxo-2'-adenosine (AOH) and/or 8-oxo-2'-*O*-methyladenosine (AmOH) were confirmed by gel retardation assays. As shown in Figure 2, the single-, double-, and triple-stranded species can be separated on a 12% polyacrylamide gel at pH 7.0. It is worth noting that a band, corresponding to a weaker binding mode of the pyrimidine oligonucleotides to the imperfect C-G sites, was observed for the oligoribonucleotide analogues lacking the complete substitution of 8-oxo-adenosine for cytidine. On the other hand, we could detect more stable triple-helical structures with the oligo-2'-*O*-methyl-ribonucleotides with from one to three AmOH substitutions.

The exonuclease resistance of the homopyrimidine oligoribonucleotides and oligo-2'-*O*-methyl-ribonucleotides containing AOH and AmOH was examined in the medium used for anti-HIV assays, which contains 10% fetal bovine serum (FBS) (Figure 3). For comparison, the unmodified oligoribonucleotide (**1**) was chosen as a control. The oligo-2'-*O*-methyl-ribonucleotide (**5**) and the oligo-2'-*O*-methyl-ribonucleotide containing AmOH (**8**) were stable (50% and 53%) after 8 h of incubation, whereas the oligoribonucleotide (**1**) and the oligoribonucleotide containing AOH (**4**) were completely degraded (100%) after 2 h. An increased resistance to nuclease degradation has been observed in oligo-2'-*O*-methyl-ribonucleotide derivatives incubated with 3'-exonucleases.[28-31] However, the results reported by Monia[29] clearly indicate that the nuclease resistance conferred on an adjacent P-O linkage by a 2'-*O*-methyl substituent still does not allow for potent antisense activity in cellular systems. On the other hand, the oligo-2'-*O*-alkyl-ribonucleotide binds complementary sequences with high affinity, relative to analogous DNA or RNA oligonucleotides. The clinical applications of oligo-2'-*O*-methoxyethyl-ribonucleotides with a phosphorothioate backbone are being explored in phase I/II clinical trials directed against PCK-α in refractory solid tumors.[32] This stabilization

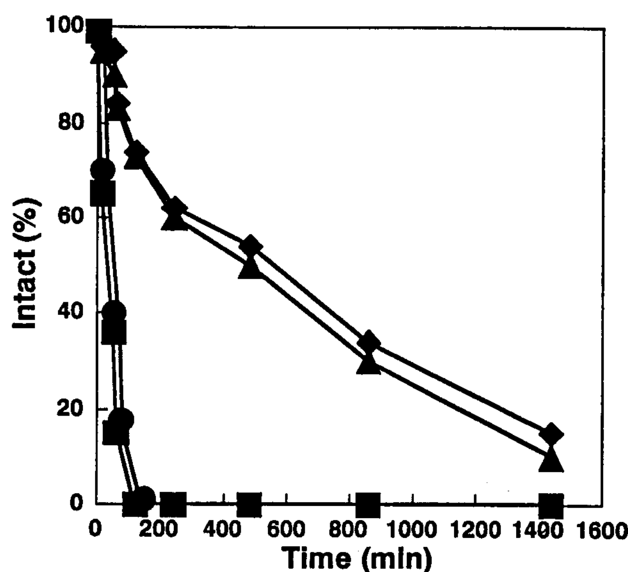


Figure 3. Stability of oligonucleotide derivatives (**1**,**4**, **5**, and **8**) in the presence of 10% calf serum. Oligonucleotides were incubated in the presence of 10% calf serum for the indicated times, as described in the Materials and Methods. Full-length ("intact") and digested oligonucleotides were resolved on 20% denaturing polyacrylamide gels, and the full-length oligonucleotide was quantitated at the indicated time points, as described in the Materials and Methods. Oligonucleotides tested are indicated as follows. ■, oligo **1**; ●, oligo **4**; ▲, oligo **5**; ◆, oligo **8**. The percentage of the oligonucleotide that remained intact was calculated by comparison with the full-length oligonucleotide levels in the samples.

should help us to design much more efficient chemical recognition enzymes, which could be used as tools *in vitro* and *in vivo.*

The restriction enzyme *Ksp* 632-I recognizes the sequence 5'-CTCTTC-3'/3'-GAGAAG-5', and cleaves the two strands asymmetrically outside the recognition sequence (Figure 1). We have synthesized homopyrimidine oligoribonucleotides and oligo-2'-*O*-methyl-ribonucleotides containing AOH and/or AmOH instead of cytidine, which are 17 bases in length and have a parallel orientation as compared to the homopurine sequence of the 17 bp SV40 DNA sequence. Previous reports showed that this oligonucleotide binds to the major groove of the duplex DNA in the homopurine sequence.[6,11]

In order to test the ability of the 17 mer homopyrimidine oligoribonucleotides and the oligo-2'-*O*-methyl-ribonucleotides containing AOH and/or AmOH instead of cytidine to recognize selectively their *Ksp* 632-I site within the SV40 DNA, the experiments were conducted under physiologically relevant pH conditions. SV40 DNA was digested with *Hpa*II (position 346) and was used as a substrate to assay the restriction enzyme cleavage activity of the homopyrimidine-oligoribonucleotide derivatives. Cleavage of linear SV40 DNA with *Ksp* 632-I yields fragments of 1150 and 4090 base pairs in length. The experiments were carried out at 30°C and at either pH 7.0 or 8.0, in the presence of 100 mM NaCl, 66 mM potassium acetate, 10 mM $MgCl_2$, and 1 mM spermine. The DNA fragments were visualized by ethidium bromide staining and were subjected to quantitative analysis, assuming that the fluorescent intensities were proportional to the lengths of the DNA fragments. The oligonucleotides were added to the SV40 linear DNA before the addition of the restriction enzyme. Figures 4A (lane 3) and 4C (lane 3) show the digestion of the SV40 DNA by *Ksp* 632-I in the presence of the oligoribonucleotides and the oligo-2'-*O*-methyl-ribonucleotides (**1 and 5**). Densitometric analysis of the gels indicated that the percentages of restriction enzyme cleavage inhibition were 35% and 10%, respectively. When the pH of the cleavage reaction was increased to 8.0, no inhibition of the restriction enzyme was observed with the oligoribonucleotide (**1**) (Figure 4B, lane 3). In the case of **5**, very weak cleavage protection was observed (Figure 4C, lane 3). This was also supported by the gel retardation assay (Figure 2). Further evidence for the oligonucleotide binding to the duplex DNA was obtained using conditions that are known to either destabilize or stabilize triple-helical structures. Triple helix formation by a homopyrimidine oligonucleotide involves binding of uridine and protonated cytidine to A-T and G-C base pairs, respectively. An increase in the pH destabilizes the triple helix, due to the loss of cytidine protonation, which is required to form the C-G-C base triplet.

In a subsequent experiment, we incubated the SV40 linear DNA with *Ksp* 632-I in the presence of the homopyrimidine oligoribonucleotides and the oligo-2'-*O*-methyl-ribonucleotides containing AOH and/or AmOH instead of cytidine (**2-4** and **6-8**). Gel analysis of the modified oligomers (**2-4** and **6-8**) revealed cleavage inhibition by 52% (**2**), 68% (**3**), 94% (**4**), 100% (**6**), 100% (**7**), and 100% (**8**) (Figures 4A (lanes 4-6) and 4C (lanes 4-6)). Furthermore, the above experiment was also repeated at pH 8.0, using the homopyrimidine oligoribonucleotides and the oligo-2'-*O*-methyl-ribonucleotides containing AOH and/or AmOH instead of cytidine (**2-4** and **6-8**). The percentages of inhibition of the restriction enzyme cleavage of the modified oligomers (**2-4** and **6-8**) were 0% (**2**), 11% (**3**), 95% (**4**), 86% (**6**), 100% (**7**), and 100% (**8**) (Figure 4B (lanes 4-6) and D (lanes 4-6)). The RNA oligonucleotides (**2** and **3**) with one and two AOH substitutions showed less inhibition of the endonuclease activity than the oligo-2'-*O*-methyl-ribonucleotides

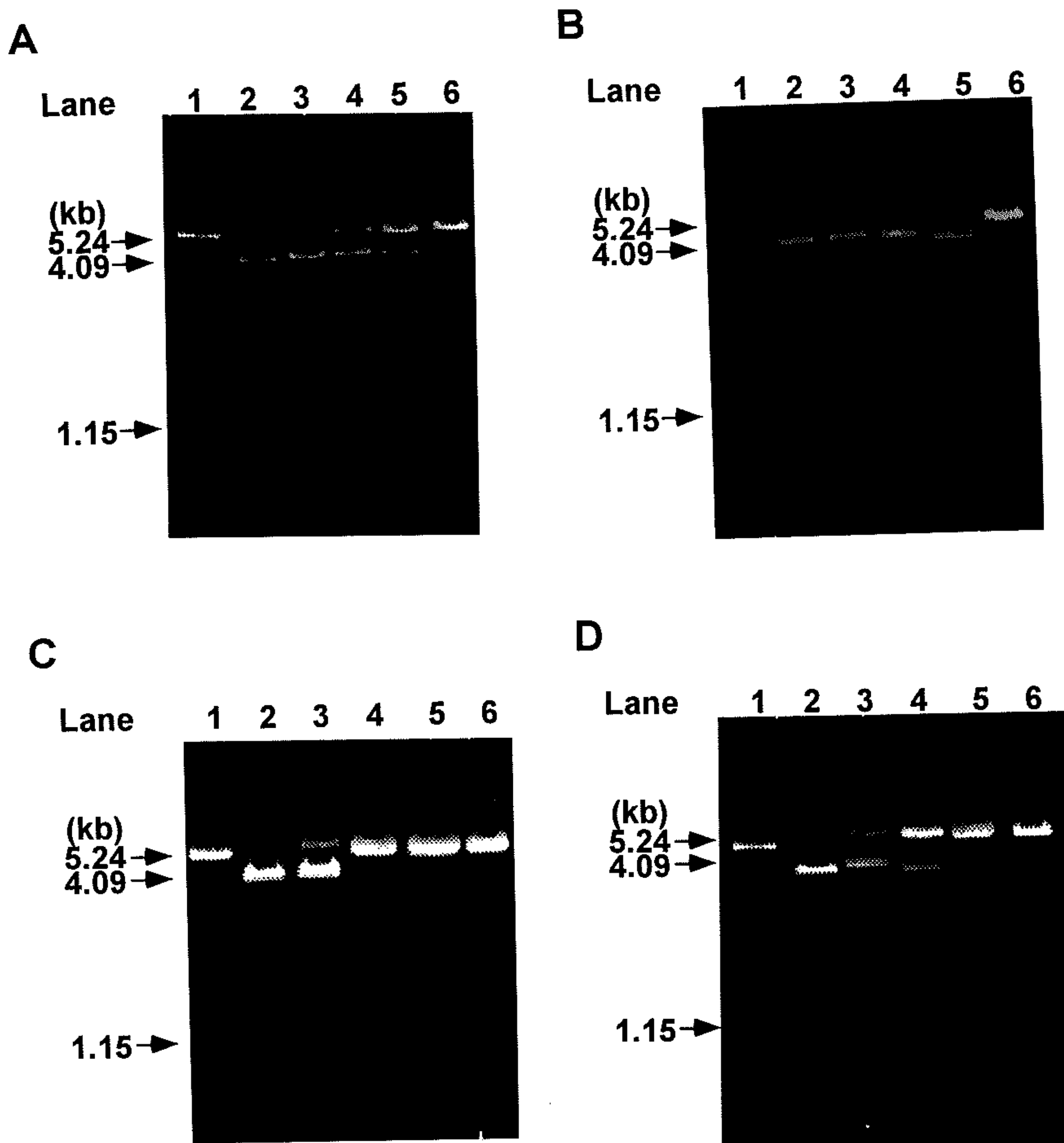


Figure 4. Specific inhibition of the restriction enzyme *Ksp* 632-I by the homopyrimidine oligonucleotides containing 8-oxo-adenosine and 8-oxo-2'-*O*-methyladenosine. The enzymatic assay was performed by incubating SV40 linear DNA (6 nM) at 30°C for 1 h with *Ksp* 632-I (20 units/μL) and the oligonucleotide in a buffer containing 33 mM Tris-acetate, 66 mM potassium acetate, 0.5 mM dithiothreitol, 10 mM $MgCl_2$, 1 mM spermine, and 1 μg/μL tRNA. A). The enzymatic assay was carried out in the presence of the unmodified (**1**) (lane 3) and modified (**2-4**) (lanes 4-6) oligonucleotides at pH 7.0. B). The enzymatic assay was carried out in the presence of the unmodified (**1**) and modified (**2-4**) oligonucleotides at pH 8.0. C). The enzymatic assay was carried out in the presence of the modified (**5-8**) oligonucleotides at pH 7.0. D). The enzymatic assay was carried out in the presence of the modified (**5-8**) oligonucleotides at pH 8.0. Lane 1: SV40 linear DNA incubated without restriction enzyme. Lane 2: SV40 linear DNA incubated with restriction enzyme.

(**6-7**) with one and two AmOH substitutions. It is notable that this potent inhibition is achieved in a modified

oligonucleotide (**8**) possessing a melting temperature of 39℃, a higher Tm than those of the other modified oligonucleotides. These results suggest that the oligo-2'-*O*-methyl-ribonucleotides with from one to three AmOH substitutions provided very high cleavage protection in the physiological pH range. In particular, in the case of the substitution of only one cytidine residue with AmOH, the inhibition of the restriction enzyme was high. In contrast, the oligoribonucleotide analogues with one or two substitutions of AOH provided only weak cleavage protection. However, in the case of the substitution of three cytidine residues with AOH, inhibition of the restriction enzyme was observed. These findings suggest that the homopyrimidine oligoribonucleotides and the oligo-2'-*O*-methyl-ribonucleotides containing AOH and AmOH can specifically inhibit the DNA-protein interaction via triplex formation, within the physiological pH range. The substitution of one cytidine residue with AmOH showed higher endonuclease inhibition than the substitution of either one or two cytidine residues with AOH. Furthermore, the oligo-2'-*O*-methyl-ribonucleotides with from one to three AmOH substitutions provided very high cleavage protection in the physiological pH range. In particular, in the case of the substitution of only one cytidine residue with AmOH, the restriction enzyme inhibition was similar to that of the oligomer (**4**).

The recognition of a DNA sequence by a restriction endonuclease can be strongly inhibited under physiological pH conditions (pH 6.0-8.0) by homopyrimidine oligo-2'-*O*-methyl-ribonucleotides containing AmOH, which bind with high affinity to duplex DNA. Specific binding of a homopyrimidine oligo-2'-*O*-methyl-ribonucleotide containing AmOH might modulate transcription, either by interacting with the binding of protein factors or by preventing the elongation of RNA. DNA replication might be similarly inhibited. The oligo-2'-*O*-methyl-ribonucleotide derivatives also display enhanced resistance to nuclease degradation by 3'-exonucleases. This stabilization should help us to design much more efficient transcription and replication inhibitors, which possess favorable pharmacological properties, thus facilitating their applications as antigene agents in animal testing and therapeutic development.

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