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Synthesis and base pairing properties of DNA-RNA heteroduplex containing 5-hydroxyuridine

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5-Hydroxyuridine (5-OHU) is a major lesion of uridine and cytosine produced in RNA by various chemical oxidants. To elucidate its biochemical and biophysical effects on RNA replication, the site-specifically modified oligoribonucleotides containing 5-OHU were synthesized with C5-hydroxy-5'-O-DMTr-2'-TBDMS-uridine phosphoramidite using automated solid phase synthesis. The base-pairing properties of nucleotides opposite 5-OHU in 24 mer oligoribonulcleotides with dNTP were studied using three reverse transcriptases (SuperScript™II-, AMV-, MMLV-RT) in cDNA synthesis. Adenine as well as guanine was incorporated preferentially by all reverse transcriptases. In the UV-melting temperature experiment, the results from the relative stabilities of the base pairs were A : 5-OHU > G : 5-OHU > T : 5-OHU ≈ C : 5-OHU. Circular Dichroism (CD) studies showed that DNA-RNA containing 5-OHU heteroduplexes exhibit a similar conformation between the A-type RNA and B-type DNA. These results suggest that 5-OHU from oxidative damage was mainly influenced by adenine mismatch. [BMB reports 2009; 42(6): 373-379]

INTRODUCTION

Oxidative damage of DNA or RNA bases by reactive oxygen species plays important roles in cellular processes and causes miscoding lesions that are potentially mutagenic, carcinogenic or generating interstrand crosslinking (1-4). Purine bases in DNA duplexes and in cellular dNTP pools are easily hydroxylated by reactive oxygen species (5, 6). Among them, 8-oxo-7, 8-dihydroxyguanine (8-oxoG) and 8-oxo-7,8-dihydroxyadenine (8-oxoA) are the most frequently occurring oxidized bases (7). Also, base residues in ribonucleotides are oxidized in cells (8, 9). Recently, several studies have revealed that pyrimidine bases are frequently modified at the 5th position during oxidation (10-12). 5-Hydroxyuridine (5-OHU) is a major oxidized nucleobase that can be generated by the action of stable OH radicals and one-electron mediated products. It has been reported that the oxidized cytosine product 5-OHU is the major precursor to the GC to AT transition mutations (10). 5-OHU is generated by oxidative deamination of cytosine and has been found at high levels in the liver, kidney, and brain DNA (13).

Unlike the DNA field of research, the base lesion in RNA strands is not well understood. In a cell, RNA is more abundant than DNA and almost all of the cellular RNAs have functional capacity for protein synthesis whether they are encoding proteins (mRNA) or involved in translation (rRNA and tRNA). However, only 28% of the human genomic DNA is transcribed into RNA, with 5% of the transcribed sequences actually encoding proteins (14). Moreover, under similar conditions, the levels of oxidative damage in RNA are usually higher than in DNA which may impair protein synthesis or other RNA functions (15). Although the importance of the base-pairing properties of 5-OHU in DNA also have been studied (16), the base pairing properties of 5-OHU in RNA were not characterized. Therefore, investigating the potential relationship between the base pairing properties of 5-OHU and RNA oxidative damage is very important.

Previously, we reported the base pairing properties of 8-oxoG and 8-oxoA in cDNA synthesis by reverse transcriptases and the thermodynamic stability of the DNA-RNA heteroduplex containing 8-oxoG (17-20). In the present study, in order to extend the understanding of the base pairing properties of 5-OHU in RNA, 24 base-long oligoribonucleotides [5'-AUGGACXGAAUUGGGUGUCGACAU-3', X=U or 5-OHU] containing 5-OHU, were prepared. The incorporation properties of DNA bases were analyzed though opposite the 5-OHU *in vitro* cDNA synthesis with Superscript™II-, MMLV- and AMV-reverse transcriptases. Thermodynamic stabilities and structures of DNA-RNA hetreoduplexes containing 5-OHU were analyzed.

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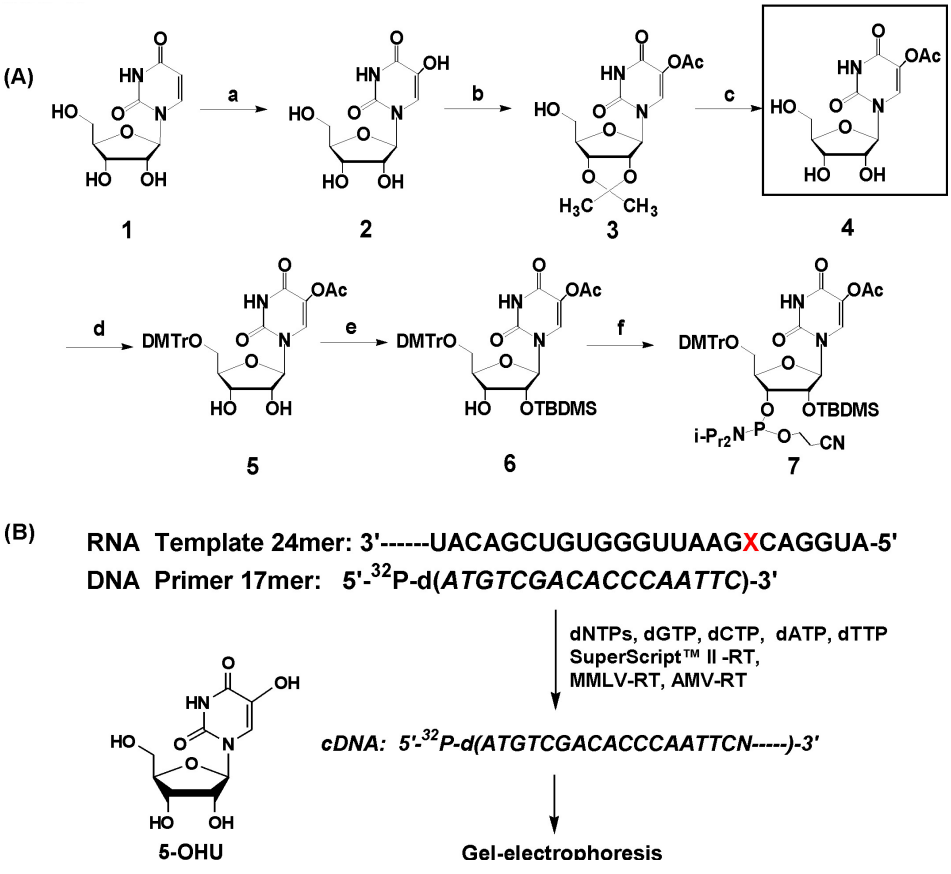
RESULTS AND DISCUSSION

Synthesis of oligoribonucleotides containing 5-OHU

The synthetic routes for the preparation of 5-OHU phosphoramidite are illustrated in Scheme 1 (A). The purities of oligoribonucleotides were evaluated to be over 97% by PAGE and HPLC. In order to identify whether the oligoribonucleotide contained 5-OHU, the presence of 5-OHU was analyzed by enzymatic digestion of oligoribonucleotides with a combination of phosphodiesterase I and alkaline phosphatase and separating the component nucleosides by reverse phase HPLC (supplementary Fig. 1. and MALDI-TOF (supplementary Fig. 2. The degradation of oligoribonucleotides also proved the presence of the modified uridine derivatives, and the distinctive mobility of 5-OHU in oligoribonucleotides was also observed in PAGE (supplementary Fig. 3).

Incorporation of a single dNTPs opposite to 5-OHU

Investigation of the incorporation of dNTPs opposite to 5-OHU during cDNA synthesis was carried out using three reverse transcriptases (SuperScript™II-, AMV-, MMLV-RT) (Scheme 1B). 5'-End labeled 17 base-long DNA primers were extended along the modified or unmodified RNA templates by reverse transcriptases and the 18th bands of the inserted dNMPs to the 3'-end primer were analyzed by PAGE (Fig. 1A). The amount of inserted dNMPs was also calculated from the radioactivity ratios of PAGE bands (Fig. 1B). As expected in the control reaction, dAMP was preferentially incorporated into the site opposite the unmodified uridine by all reverse transcriptases compared to other dNMPs. For the site opposite to 5-OHU, dAMP was also preferentially incorporated compared to other dNMPs by reverse transcriptases. However, dGMP was also incorporated in a substantial amount by reverse transcriptases at the site opposite to 5-OHU. The amount of dGMP incorporation was dependent on the reverse transcriptases. MMLV-reverse transcriptase only incorporated dGMP up to one third of the dAMP levels and SuperScript™II- reverse transcriptase up to one forth of the dAMP levels. AMV-reverse transcriptase incorporated the least dGMP. No substantial amount of dCMP and dTMP was incorporated by any reverse transcriptase (Fig. 1B). The preferential incorporation of dAMP over dGMP at the site opposite 5-OHU by reverse transcriptases in cDNA synthesis does not agree with what is reported about DNA polymerase. Recently, several groups reported about the base-pairing properties, thermal stability



Scheme 1. (A) Synthesis of 5-hydroxyuridine amidite and its reaction conditions. (a) Br_2/H_2O, pyridine, 30 min at 0°C, 81%, (b) I. *p*-toluenesulfonic acid in aceton/2,2-dimethoxypropane; ii. $NaHCO_3$ in H_2O; 90%, (c) 10% aqueous acetic acid, 6 h at 90°C, 55% (d) DMT-Cl, DMAP in pyridine, 85%, (e) $AgNO_3$, pyridine, TBDMS- Cl in THF, 51%, (f) 2-cyanoethyl-N,N-diisopropylaminochlophophine, diisopropyl-ethylamine in CH_2Cl_2, 90%. (B) Synthesis of cDNA by reverse transcriptases where, X=U or 5-OHU. The structure of 5-OHU is shown on left.

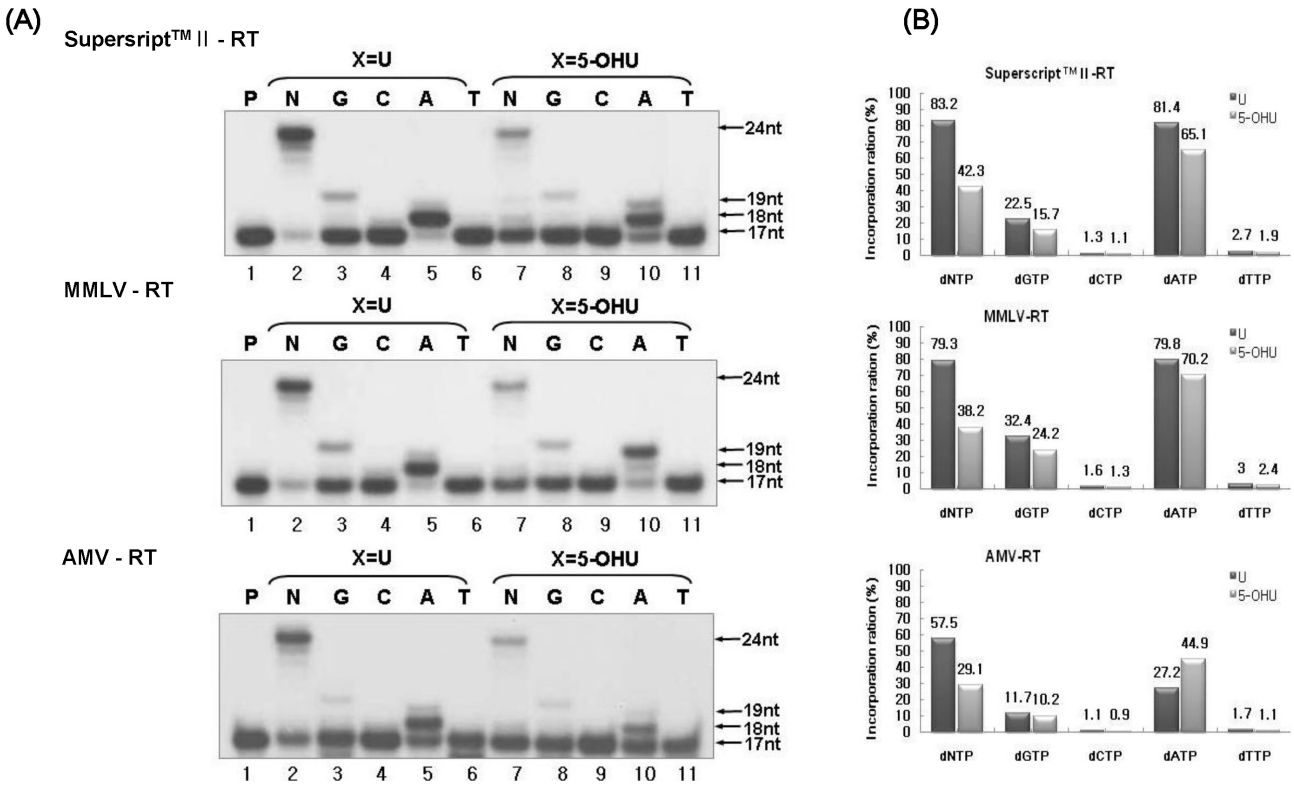


Fig. 1. Autoradiograms of polyacrylamide gels showing incorporations of dNTPs into the primer at the site opposite U and 5-OHU template. (A) The primer (lane 1) was incubated with Superscript™II-RT (upper), MMLV-RT (middle) or AMV-RT (lower) in the presence of dNTPs (lanes 2-7), dGTP (lanes 3-8), dCTP (lanes 4-9), dATP (lanes 5-10) and dTTP (lanes 6-11). 17 mers indicate unextended primers and 18 mers and 24 mers indicate extended primers. (B) Ratio of incorporation of dNTPs to the primers by Supersript™ II-RT, MMLV-RT and AMV-RT. The ratio (%) was calculated as Ratio = (radioactivity of newly generated spots)/(radio activity of the remaining primer + radioactivity of newly generated spots) ×100.

and the structure of modified pyrimidines (21, 22). Thiviyanathan *et al*. showed that 5-OHU would form the most stable pair with G in DNA duplex and certain DNA polymerases preferentially incorporate G opposite to 5-OHU over A during DNA replication (23). However, Volk *et al*. reported that 5-formyluracil:A base pair is more stable than the 5-formyluracil:G base pair (24).

The results of this study are somewhat different from those of 5-OHU in DNA. The base pairing properties of 8-oxoG showed a preferential pairing with dA by DNA polymerases in DNA replication but paired with dC preferentially by reverse transcriptases in cDNA synthesis (17-20). Therefore, the base incorporation property of oxidized purine and oxidized pyrimidine bases seems to depend on the enzymes catalyzed and the structure of duplexes formed.

UV melting studies of the DNA-RNA heteroduplex

The melting temperature experiment was performed in order to finding the incorporation properties of 5-OHU with dGMP and dAMP. The dodecaribonucleotides containing 5-OHU were prepared and 12 mer RNA-DNA heteroduplexes (Table 1) were constructed. Values of T_m for heteroduplexes of DNA-unmodified RNA were higher than those of heteroduplexes of DNA-modified RNA, respectively, which was expected. T_m values for heteroduplex containing 5-OHU:dA were higher than that of the heteroduplex containing 5-OHU:dG. Melting temperature analysis showed that 5-OHU formed with the most stability base pairs with dA and then next with dG. This

Table 1. Melting temperatures of DNA-RNA heteroduplexes. RNA, 5'-(AUGGACX- GAAUU)-3'; DNA, 3'-d(TACCTGYCTTAA)-5' where X = U or 5-OHU; Y = dA, dG, dC or T

Duplex	T_m (°C)	Duplex	T_m (°C)
U : dA	48.9	5-OHU : dA	42.1
U : dG	41.3	5-OHU : dG	38.9
U : dC	34.9	5-OHU : dC	34.3
U : T	38.7	5-OHU : T	34.5

The measurement was performed in a solution of 10 mM sodium cacodylate (pH 7.0), 100 mM Na^+ and 10 mM Mg^{2+}.

result is supported by the base-incorporation results in our cDNA synthesis by reverse transcriptases experiments. Stability of base-pair formation may be a significant factor in determining which base opposite to the 5-OHU residue is incorporated by reverse transcriptases. Thiviyanathan et al. reported that T_m values of DNA duplex containing 5-OHU:dG is higher than that of DNA duplex containing 5-OHU:dA (16).

CD spectroscopy

CD studies of DNA-RNA heteroduplexes with various base pairs of 5-OHU were measured at low salt conditions. It is known that the conformations of DNA-RNA heteroduplexes lie between the conformations of A-type RNA and B-type DNA (25, 26). The CD spectral patterns of DNA-RNA heteroduplexes containing U : dA, 5-OHU : dA, 5-OHU : dG, 5-OHU : T pairing were similar and the duplexes seem to adopt a conformation between A-type RNA duplex and B-type DNA duplex (Fig. 2). The duplexes containing 5-OHU : dA, 5-OHU : dG, 5-OHU : T showed a small red shift between 250-280 nm as seen in the spectra of the DNA-RNA heteroduplexes containing 8-oxoG (20).

In summary, the DNA-RNA heteroduplexes containing 5-OHU were synthesized. Base incorporation properties of dNMP opposite 5-OHU by reverse transcriptases showed that the order of incorporation was dAMP > dGMP ≫ TMP, dCMP. Melting temperature experiments showed that the relative stabilities of base pairs in DNA-RNA heteroduplexes are A : 5-OHU > G : 5-OHU > T : 5-OHU ≈ C : 5-OHU. The results of this study showed that 5-OHU would form very stable base-pairs with both A and G without causing significant perturbation in the geometry of the DNA-RNA heteroduplex. These results are not of a similar nature with the incorporation properties and thermal stabilities of the base pairing of 5-OHU in DNA. These differences in base incorporation and in thermal stability of 5-OHU in DNA and in RNA are probably due to the different enzymes used and the structure of duplexes formed respectively.

MATERIALS AND METHODS

Materials

Oligoribonucleotides were synthesized using an Applied Biosystems Incorporated 391 DNA synthesizer (ABI 391 PCR-MATE). An oligopurification cartridge (OPC) and other chemicals for oligonucleotide synthesis were purchased from Cruachem Co. (Glasgow, Scotland). SuperScript^{TM}II-, AMV, MMLV reverse transcriptase and RNase inhibitor were purchased form Invitrogen (Carlsbad, USA) and [γ-^{32}P] ATP (~3000 Ci/mmol), dATP, dGTP, dCTP, dTTP were purchased from Amersham Pharmacia Biotech (Seoul, Korea). An Applied Biosystems HPLC system (Foster, USA) equipped with an absorbance detector (Model 783A) and gradient pump system (Model 400) was employed. UV absorbance was measured using a Cary

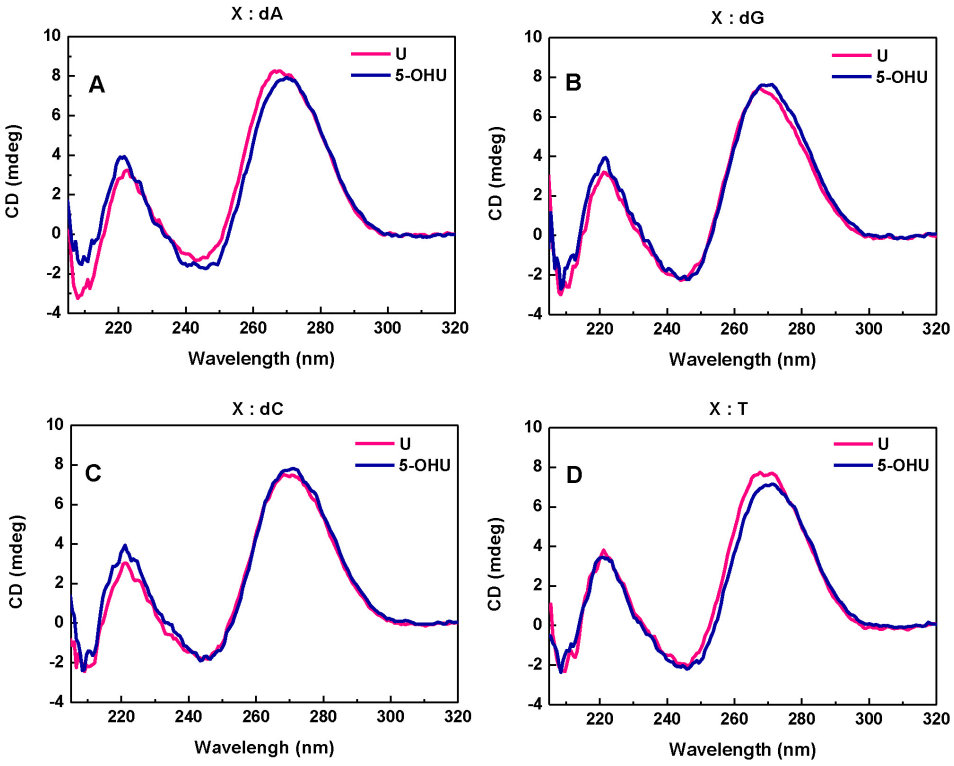


Fig. 2. CD spectra of DNA-RNA heteroduplexes. RNA: 5'-(AUGGACX GAAUU)-3'; DNA: 3'-d(TACCTGYC TTAA)-5' where X = U (---) or 5-OHU (---); Y = dA, dG, dC or dT; (A) X : dA, (B) X : dG, (C) X : dC, (D) X : T. The measurement was performed in a solution of 10 mM sodium cacodylate (pH 7.0), 100 mM Na^+ and 10 mM Mg^{2+} at 25°C.

1-Bio spectrophotometer (Varian Associates) and circular dichroism spectra were measured using a Jasco J810 spectrophotometer.

Synthesis of 5-OHU building blocks

As shown in scheme 1 (A), compound 2 was prepared via bromination followed by hydrolysis in water from compound 1 which was commercially available (Tokyo Chemical Industry Co. Japan) (27). After protecting the two hydroxyl groups at 2'- and 3'-positions using isopropylation in acetone containing 2,2-dimethoxypropane, the hydroxyl group at the 5-position was acetylated in an aqueous medium to produce compound 3 (28). In the following step, compound 3 was treated with aqueous acetic acid to produce the hydrolyzed product 4 (29).

Compound 4 5-OHU-Ac (0.40 g, 1.64 mmol) and 4-(dimethylamino) pyridine (*DMAP*) (0.025 g, 0.20 mmol, 12 mol%) were dissolved in pyridine (10 ml), and 4,4'-dimethoxytrityl (*DMT*) chloride (0.83 g, 2.46 mmol, 150 mol%) was added. After 1.5 h at room temperature, additional DMT-Cl (0.50 g, 1.48 mmol, 90 mol%) was added. After 3 h, the solvent was removed in vacuum, and the residue was redissolved in CH_2Cl_2 (50 ml) and washed with $NaHCO_3$ (20 ml) and NaCl (20 ml). The organic solution was dried by anhydrous Na_2SO_4, filtered, and concentrated. The residue was subjected to chromatography (8% MeOH/CH_2Cl_2) to yield 5-acetoxy-5'-O-(4,4'-dimethoxytrityl) uridine (compound 5) 85%. ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 11.80 (br, 1H, NH), δ 7.78 (s, 1H, H6), δ 7.40-6.86 (m, 13H, DMT), δ 5.78 (d, 1H, H1'), δ 5.55 (d, 1H, H2'), δ 5.19 (d, 1H, H3'), δ 4.17 (m, 2H, H5'), δ 3.98 (d, 1H, H4'), δ 3.74 (s, 6H, DMT), δ 1.91 (s, 3H, Ac).

Compound 5 (1.35 g, 2.23 mmol) was dissolved in tetrahydrofuran (THF, 10 ml). To this solution, silver nitrate (0.57 g, 1.5 eg) and pyridine (0.66 ml, 3.7 eg) were added. The solution was stirred until the silver nitrate was completely dissolved, then *tert*-butyldimethylsilyl (*TBDMS*) chloride (0.571 g, 1.5 eg) was added, and the solution was stirred at room temperature for 7 h. The different mobilities of the 2'- and 3'-silylated isomers were shown on TLC. After work-up, the 2'-silylated isomer was separated by column chromatography on silica-gel to yield 51%. ^{1}H NMR (400 MHz; DMSO-d_6; TMS) δ 11.52 (br, 1H, NH), δ 7.69 (s, 1H, H6), δ 7.29-6.79 (m, 13H, DMT), δ 5.72 (d, 1H, H1'), δ 5.09 (d, 1H, H3'), δ 4.22 (d, 1H, H4'), δ 4.02 (m, 2H, H5'), δ 3.66 (s, 6H, DMT), δ 1.79 (s, 3H, Ac), δ 0.83 (s, 9H, *t*-Bu), δ 0.01 (s, 3H, CH_3), δ -0.01 (s, 3H, CH_3).

Phosphitylation of the protected nucleosides was accomplished by treatment with 2-cyanoethyl-N,N-diisopropylaminochlorophosphine and triethylamine in THF. After purification, the phosphoramidite units were precipitated in toluene-petroleum ether at −20°C (30).

Synthesis and identification of oligonucleotides

Dodecaribonucleotides were synthesized using standard solid phase phosphoramidite chemistry on an automated oligonucleotide synthesizer using slightly modified protocols and 4,5-dicyanoimidazole as a coupling agent. The oligonucleotides were cleaved from the solid support and deprotected using 3 : 1 (v/v) ammonia/ethanol at 55°C for 18 h. The mixture was then concentrated and dried in a lyophilizer. The 2' hydroxyl group was deprotected by overnight incubation with 1 M tetrabutylammonium fluoride for 24 h, followed by *n*-butanol precipitation. The crude oligonucleotides were purified by an OPC and 20% polyacrylamide gel electrophoresis containing 8 M urea. Extracted oligonucleotides from gel slices were desalted by reverse phase HPLC. Dodecadeoxynucleotides sequences complementary to the dodecaribonucleotides were also prepared using standard solid phase phosphoramidite chemistry. The purities of dodecaribonucleotides were identified by 20% denaturating polyacrylamide gel electrophoresis 8 M urea and by reverse phase HPLC.

Radiolabeling of oligonucleotides

Oligonucleotides (50 pmol) were labeled at the 5'-end with 10 μCi of [γ-^{32}P]-ATP (2 pmol, 10 mCi/mL) upon incubation with T4 polynucleotide kinase (10 units) in 10 μL of supplied buffer at 37°C for 30 min. Then, the reaction was stopped by addition of 1 μL of a 0. 5 M EDTA solution (pH 8.0). Unincorporated [γ-^{32}P]-ATP was removed by purification of the oligonucleotides on PAGE.

Synthesis of cDNA by reverse transcriptases

Transcriptions catalyzed by SuperScript™II-RT, MMLV-RT and AMV-RT were carried out in 10 μl solutions containing commercially presented 5× reverse transcription buffer (40 mM Tris-HCl (pH 8.0), 10 mM DTT, 100 mM KCl, 2.5% glycerol and 5 mM $MgCl_2$), [γ-^{32}P]ATP (5,000 cpm) 5' end-labeled, unlabelled 17 mer DNA primer (100 μM) and 24 mer RNA template (150 μM). These solutions were heated at 80°C for 5 min then cooled to room temperature over a period of 3 h. Primer extension was carried out in a volume of 20 μl containing annealed template and primer hybrid solutions, DTT (100 mM), 2.5 μM of dATP, dGTP, dCTP, or dTTP, or mixed dNTPs, Rnase inhibitor (20 units), and reverse transcriptases (20 units). After incubation at 39°C for 2 h, the reactions were extracted with phenol/chloroform, and then 10 μl of the aqueous aliquots with 10 μl loading buffer (9 M urea, 5 mM Tris-borate (pH 8.3), 1 mM EDTA, 0.02% XC and 0.02% BPB) were loaded onto 20% PAGE (31). Transcription and extension products were electrophoresed by 20% PAGE 7 M urea at 1,000 V for 3 −5 h and were exposed to X-ray film at −70°C for 12 h. The radio-activities in gel bands corresponding to the products were analyzed by a Gel Documentation System and Labwork™ software.

DNA-RNA heteroduplex UV-melting experiments

Melting curves were recorded at 260 nm using a Cary 1-Bio spectrophotometer (Varian Associates) equipped with a temperature control. Oligoribonucleotide (2 μM) and a complementary ODN (2 μM) for the melting experiments were prepared

in a buffer containing 10 mM sodium cacodylate (pH 7.0), 100 mM NaCl and 10 mM $MgCl_2$. Each sample was heated at 80°C for 5 min and then the solution cooled gradually to an appropriate temperature and used for the thermal denaturation studies. UV absorbance at 260 nm was monitored every 1°C from 0°C to 70°C. Heating rate was set at 1°C/min. Reported parameters are the average of three independent experiments.

DNA-RNA heteroduplexes CD experiments

CD measurements were conducted on a Jasco J-810 spectropolarimeter equipped with a variable Peltier temperature controller. All CD experiments were conducted at 15°C. Typically, oligoribonucleotide (2 μM) and a complementary ODN (2 μM) of a duplex sample were dissolved in 1 ml of a buffer containing 10 mM sodium cacodylate (pH 7.0), 100 mM NaCl and 10 mM $MgCl_2$, and placed in a 10 mm path length cell. The sample was heated at 80°C for 5 min and then cooled to 15°C over a 10 min period. The spectropolarimeter scanned from 205 to 320 nm at a rate of 50 nm/min. Data points were acquired every 0.2 nm with a 2 s response time. Three scans were collected for each sample.

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REFERENCES

1. Fraga, C. G., Shigenaga, M. K., Park, J. W., Degan, P. and Ames, B. N. (1990) Oxidative damage to DNA during aging: 8-Hydroxy-2'-Deoxyguano-sine in rat organ DNA and urine. *Proc. Natl. Acad. Sci. U.S.A.* **87,** 4533-4537.
2. Poulsen, H. E., Prieme, H. and Loft, S. (1998) Role of oxidative DNA damage in cancer initiation and promotion. *Eur. J. Cancer Prev.* **7,** 9-16.
3. Friedberg, E. C., McDaniel, L. D. and Schultz, R. A. (2004) The role of endogenous and exogenous DNA damage and mutagenesis. *Curr. Opin. Genet. Dev.* **14,** 5-10.
4. Rahman, I. (2003) Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. *J. Biochem. Mole. Biol.* **36,** 95-109.
5. Shibutani, S., Takesita, M. and Grollman, A. P. (1991) Insertion of specific bases during DNA synthesis past the oxidationdamaged base 8-oxodG. *Nature* **349,** 431-434.
6. Shibutani, S., Bodepudi, V., Johnson, F. and Grollman, A. P. (1993) Translational synthesis on DNA templates containing 8-oxo-7,8-dihydroadenosine. *Biochemistry* **32,** 4615-4621.
7. Tan, X., Grollman, A. P. and Shibutani, S. (1999) Comparison of the mutagenic properties of 8-oxo-7,8-dihydro-2'-deoxyadenosine and 8-oxo-7-8-dihydro-2'-deoxyguanosine DNA lesions in mammalian cells. *Carcinogenesis* **20,** 2287-2292.
8. Rhee, Y. S., Valentine, M. R. and Termini, J. (1995) Oxidative base damage in RNA detected by reverse transcriptase. *Nucleic. Acids. Res.* **23,** 3275-3282.
9. Duarte, V., Muller, J. G. and Burrows, C. J. (1999) Insertion of dGMP and dAMP during *in vitro* DNA synthesis opposite an oxidized form of 7,8-dihydro-8-oxoguanine. *Nucleic. Acids Res.* **27,** 496-502.
10. Feig, D. I., Sowers, L. C. and Loeb, L. A. (1994) Reverse chemical mutagenesis: identification of the mutagenic lesions resulting from reactive oxygen species-mediated damage to DNA. *Proc. Natl. Acad. Sci. U.S.A.* **91,** 6609-6613.
11. Kreutzer, D. A. and Essigmann, J. M. (1998) Oxidized, deaminated cytosines are a source of C → T transitions *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* **95,** 3578-3582.
12. Vaisman, A. and Woodgate, R. (2001) Unique misinsertion specificity of poliota may decrease the mutagenic the potential of deaminated cytosines. *EMBO J.* **20,** 6520-6529.
13. Wagner, J. R., Hu, C. and Ames, B. N. (1992) Endogenous oxidative damage of deoxycytidine in DNA. *Proc. Natl. Acad. Sci. U.S.A.* **89,** 3380-3384.
14. Brégeon, D. and Sarasin, A. (2005) Hypothetical role of RNA damage avoidance in preventing human disease. *Mutat. Res.* **577,** 293-302.
15. Li, Z. W., Wu, J. H. and Deleo, C. J. (2006) RNA damage and surveillance under oxidative stress. *IUBMB. Life* **58,** 581-588.
16. Volk, D. E., Thiviyanathan, V., Somasunderam, A. and Gorenstein, D. G. (2006) Ab initio base-pairing energies of uracil and 5-hydroxyuracil with standard DNA bases at the BSSE-free DFT and MP2 theory levels. *Org. Biomol. Chem.* **4,** 1741-1745.
17. Kim, S. K., Yokoyama, S., Takaku, H. and Moon, B. J. (1998) Oligoribonucleotides containing 8-oxo-7,8-dihydro-guanosine and 8-oxo-7,8-dihydro-2'-O-methylguanosine: synthesis and base pairing properties. *Bioorg. & Med. Chem. Lett.* **8,** 939-944.
18. Kim, S. K., Kim, J. Y., Yokoyama, S., Takaku, H. and Moon, B. J. (1999) Misreading of templates containing 8-oxo-7,8-dihydroguanosine or 8-oxo-2'-O-methylguanosine in cDNA synthesis by reverse transcriptases. *Nucelosides Nucleotides* **18,** 1335-1337.
19. Kim, S. K., Kim, J. Y., Baek, A. K. and Moon, B. J. (2002) Base pairing properties of 8-oxo-7,8-dihydroadenosine in cDNA synthesis by reverse transcriptases. *Bioorg. & Med. Chem. Lett.* **12,** 1977-1980.
20. Kim, S. K., Lee, S. W., Kwon, O. S. and Moon, B. J. (2004) DNA·RNA heteroduplex containing 8-oxo-7,8-dihydroguanosin: base pairing, structures and thermodynamic stability. *J. Biochem. Mole. Biol.* **37,** 657-662.
21. Ahmadian, M., Zhang, P. and Bergstrom, D. E. (1998) A comparative study of the thermal stability of oligodeoxyribonucleotides containing 5-substituted 2'-deoxyuridines. *Nucleic. Acid. Res.* **26,** 3127-3135.
22. Haeberli, P., Berger, I., Pallan, P. S. and Egli, M. (2005) Syntheses of 4'-thioribonucleosides and thermodynamic stability and crystal structure of RNA oligomers with incorporated 4'-thiocytosine. *Nucleic. Acid. Res.* **33,** 3965-3975.
23. Thiviyanathan, V., Somasunderam, A., Volk, D. E., Hazra, T. K., Mitra, S. and Gorenstein, D. G. (2008) Base-pairing properties of the oxidized cytosine derivative, 5-hydroxy uracil. *Biochem. Biophys. Res. Commun.* **366,** 752-757.
24. Volk, D. E., Thiviyanathan, V., Somasunderam, A. and

Gorenstein, D. G. (2007) Ab initio base-pairing energies of an oxidized thymine product, 5-formyluracil, with standard DNA bases at the BSSE-free DFT and MP2 theory levels. *Org. Biomol. Chem.* **5,** 1554-1558.
25. Wang, L. and Keiderling, T. A. (1992) Vibrational circular dichroism studies of the A-to-B conformational transition in DNA. *Biochemistry* **31,** 10265-10271.
26. Cummins, L. L., Owens, S. R., Risen, L. M., Lesnik, E. A., Freier, S. M., McGee, D., Guinosso, C. J. and Cook, P. D. (1995) Characterization of fully 2'-modified oligonucleotide hetero- and homoduplex hybridization and nuclease sensitivity. *Nucleic. Acids Res.* **23,** 2019-2024.
27. Fujimoto, J., Tran, L. and Sowers, L. C. (1997) Synthesis and cleavage of oligodeoxynucleotides containing a 5-hydroxyuracil residue at a defined site. *Chem. Res. Toxicol.* **10**, 1254-1258.
28. Rabi, J. A. and Fox, J. J. (1972) Nucleosides. LXXVI. A synthesis of a carbon carbon bridged pyrimidine cyclonucleoside. *J .Org. Chem.* **37,** 3898-3901.
29. Ferris, J. P., Ramachandra, R. V. and Newton T. A. (1979) Nucleoside synthesis by the photorearrangement of ribofuranosyl enamino nitriles. *J. Org. Chem.* **44,** 4378-4381.
30. Scaringe, S. A., Franklyn, C. and Usman, N. (1990) Chemical synthesis of biologically active oligoribonucleotides using β-cyanoethyl protected ribonucleoside phosphoramidites. *Nucleic. Acid Res.* **18,** 5433-5441.
31. Kim, Y. H., Hong, Y. B., Suh, S. W. and Jung, G. U. (2000) Nucleotide insertion fidelity of human hepatitis B viral polymerase. *J. Biochem. Mole. Biol.* **33,** 126-132.