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Misreading of RNA Templates Containing 8-Oxo-7,8-Dihydroguanosine Or 8-Oxo-2'-O-Methylguanosine in cDNA Synthesis by Reverse Transcriptases

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MISREADING OF RNA TEMPLATES CONTAINING 8-OXO-7,8-DIHYDROGUANOSINE OR 8-OXO-2'-O-METHYLGUANOSINE IN cDNA SYNTHESIS BY REVERSE TRANSCRIPTASES

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ABSTRACT: RNA template 30mers which contain 7,8-dihydroguanosine(8-oxoG) or 7,8-dihydro-2'-O-methylguanosine(8-oxo-G-Me) were chemically synthesized. ³²P-Labeled DNA primer 19mer was extended with dNTPs by MMLV and AMV reverse transcriptases on the RNA templates. Incorporation of nucleotides opposite 8-oxoG and 8-oxoG-Me were investigated.

Deoxyguanosine residues in DNA are hydroxylated at the C8 position both *in vivo* and *in vitro* to form an 8-oxo-7,8-dihydroxydeoxyguanosine(8-oxodG) by oxidative stress¹. The formation of 8-oxodG is considered to be a likely cause of various types of cellular DNA damage including strand breaks, abasic sites and base modifications. Some of this cellular DNA damage is suspected to play an important role in mutagenesis, carcinogenesis, aging and other degenerative diseases^{1,2}. Oxidative base damages in RNA strands were also analyzed in the oxidoreduction of *Torula* yeast RNA³, and also revealed oxidatively damaged sites using DNA primer extension assay with AMV reverse transcriptase⁴. It has been reported that 8-oxoguanosine(8-oxoG) was formed in isolated RNA bacteriophages which was photoinactivated with methylene blue and rose bengal⁵. Contrast to well characterization of the base pairing, misreading and conformation of the 8-oxodG, little is known to the those of the 8-oxoG.

In order to study, the structure and base pairing properties of 8-oxoG, we synthesized RNA template [5'-UCC AUU UUC AXA AUU GGG UGU CGA CAU AGC-3'(X=8-oxoG and 8-oxoG-Me) 30mers via phosphoramidite method⁶. ³²P-Labeled DNA primer 19mer was extended with dNTPs by MMLV and AMV reverse transcriptase on the RNA

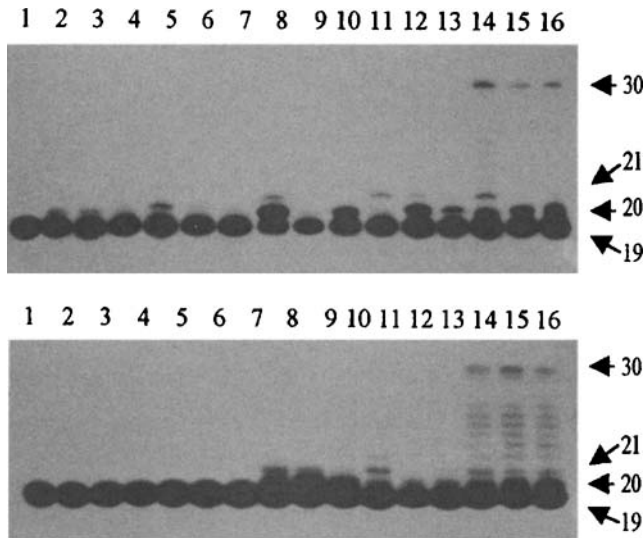


FIG.1. Autoradiograms of polyacrylamide gels showing incorporations of dNTPs into the primer at the site opposite to X ; the normal template ORNs-1(X=G, lane 2, 5, 8, 11, 14), modified template ORNs-2(X=8-oxoG, lane 3, 6, 9, 12, 15) or ORNs-3(X= 4, 7, 10, 13, 16) and the primer(lane 1) were incubated with *MMLV-RT*(upper) or *AMV-RT*(lower) in the presence of dATP(lane 2-4), dGTP(lane 5-7), dCTP(lane 8-10), TTP(lane 11-13) and dNTPs(lane 14-16). 19mers indicate unextended primer and 20mers, 21mers and 30mers indicate extended primers.

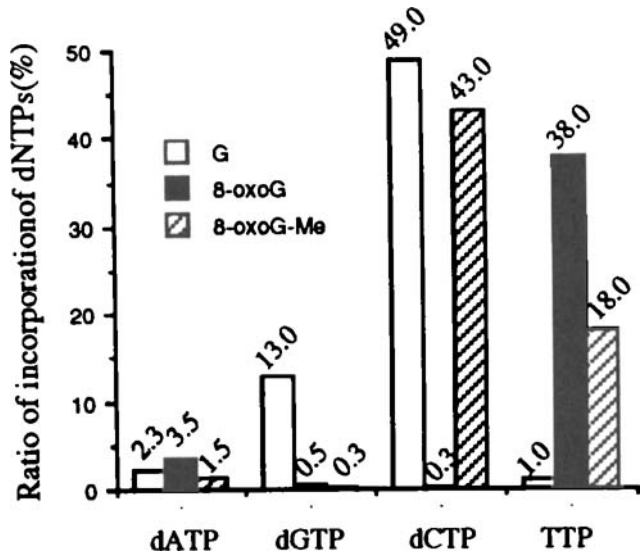


FIG.2. Ratio of incorporation of dNTPs to the primer by *MMLV-RT*

templates. Incorporation of nucleotides opposite 8-oxoG and 8-oxoG-Me in cDNA synthesis were analyzed. RNA template [5'-UCC AUU UUC AXA AUU GGG UGU CGA CAU AGC-3'(X=8-oxoG and 8-oxoG-Me) and primer, 5'd(³²P-GCT ATG TCG ACA CCC AAT T)3' were incubated with MMLV reverse transcriptase in the presence of each dNTP(Fig 1). 8-OxoG residue itself directed the insertion of dT instead of dC(Fig 2). However, when AMV reverse transcriptase was used, the 8-OxoG residue directed the insertion of the correct dC. The 8-OxoG-Me residue preferred the insertion of dC with both MMLV and AMV reverse transcriptases.

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REFERENCES

1. Ames, B.N. *Science* **1983**, 221, 1256.
2. Floyd, R.; Watson, J.; Harris, J.; West, M. and Wong, P.(1986) *Biochem. Biophys. Res. Commun.* 137, 841.
3. Yanagawa, H.; Ogawa, Y. ; Ueno, M.J. *Biol. Chem.* **1992**, 267, 13320.
4. Rhee, Y., Valentine, M.R. and Termini, J. *Nucleic Acid Res.*, **1995**, 23, 3275
5. Schneider, J.E.; Phillips, J.R.; Pye, Q.; Maitt, M.L.; Price, S.; Floyd, R. A. *Archives Biochem. Biophys.* **1993**, 301, 91.
6. Kim, S.; Yokoyama, S.; Takaku, H.; Moon, B. *Bioorg. & Medicinal Chem. Lett.* , **1998**, 8, 939.