Free Rad. Res., Vol. 23, No. 3, pp. 255–258
Reprints available directly from the publisher
Photocopying permitted by license only

FREE RADICAL DNA ADDUCT 8-OH-DEOXYGUANOSINE AFFECTS ACTIVITY OF *HPA* II AND *MSP* I RESTRICTION ENDONUCLEASES

PATRICK W. TURK and SIGMUND A. WEITZMAN*

Division of Hematology/Oncology, Department of Medicine and Robert Lurie Cancer Center, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611, USA

(Received January 16th, 1995; in revised form, February 6th, 1995)

8-OH-deoxyguanosine can diminish the ability of the restriction endonucleases *Hpa* II and *Msp* I to cleave DNA. The exact position of the adduct within the recognition site appears to determine the extent of the effect.

KEY WORDS: Reactive oxygen species, 8-OH-deoxyguanosine, 8-hydroxyl-2'-deoxyguanosine, 8-hydroxyguanine, restriction endonuclease.

Reactive oxygen species produce a wide array of damage to cells at the molecular level[1-6], and have been suggested to be a significant factor both in carcinogenesis and the aging process[7-13]. 8-OH-deoxyguanosine (8-OH-dG) is one of the more common adducts resulting from both exogenous and endogenous oxidative damage to DNA[14-20], and has been shown to affect the specificity of both prokaryotic[18] and human DNA methyltransferases (*Carcinogenesis*, in press). In this study, the presence of 8-OH-dG at the 5' and especially the 3' guanine of a 5'-CCGG-3' *Hpa* II/*Msp* I recognition site reduces the ability of these enzymes to cleave the DNA. Inhibition of cleavage by 8-OH-dG has been previously observed for the enzymes *Nhe* I[21] and *Apa* LI[22].

Synthetic oligonucleotides containing the *Hpa* II/*Msp* I recognition site were employed as substrates in restriction assays (see Figure 1). As previously described[18], oligonucleotides containing 8-OH-dG at specific positions were kindly provided by Dr. Francis Johnson[23]. Two of these oligonucleotides also contained 5-methylcytosine at specific sites. Other oligonucleotides were synthesized with either native sequences or sequences containing one or more 5-methylcytosines.

Oligonucleotides were annealed in pairs and approximately 40 pmoles (~500 ng) of each pair was digested for 24 hours at room temperature to avoid the possibility of strand denaturation at higher temperatures. Oligonucleotides were cut with either *Hpa* II (New England Biolabs, 90 units) or *Msp* I (New England Biolabs, 100 units) according to the manufacturer's buffer conditions, or not digested (control). Based upon the unit definition of each enzyme, the number of enzyme recognition sites per oligonucleotides pair (one) and the amount of oligonucleotide digested, these amounts of enzyme represent more than twenty times that required to theoretically cleave all of the sites. Aliquots from each digest were 5'-end labeled using γ-^{32}-P-ATP (Amersham Corporation) and polynucleotide kinase (United States Biochemicals), and

*To whom correspondence should be addressed

A 5′-GTACC^{M}C^{OH}GGTGACACACC-3′

B 5′-GTACC^{M}CG^{OH}GTGACACACC-3′

C 5′-GTACCC^{OH}GGTGACACACC-3′

D 5′-GTACCCG^{OH}GTGACACACC-3′

U 5′-GTACCCGGTGACACACC-3′

U′ 5′-TGGTGTGTCACCGGGTA-3′

M 5′-CTCTGAAGTACC^{M}CGGTGACACACCA-3′

M′ 5′-TGGTGTGTCAC^{M}CGGGTA-3′

M2 5′-CTCTGAAGTAC^{M}C^{M}CGGTGACACACCA-3′

M2′ 5′-TGGTGTGTCA^{M}C^{M}CGGGTA-3′

FIGURE 1 Oligonucleotide Sequences

electrophoresed through 20% polyacrylamide gels. Gels were subsequently wrapped and exposed to film at room temperature for approximately 10–20 minutes. Densitometry of films for quantification of digestion was performed using an LKB Ultroscan XL Laser Densitometer. Each digestion assay was repeated 2–3 times to confirm the accuracy of the results. The results of the restriction assays and the sequence motifs of the recognition sites are presented in Table I.

TABLE I
Effect of 8-OH-dG on *Hpa* II and *Msp* I DNA Cleavage

Oligo Pair	*Motif*	*Hpa* II Cleavage (%)	*Msp* I Cleavage (%)
U/U′	CCGG GGCC	100	100
U/M′	CCGG GGMC	0	100
M/M′	CMGG GGMC	0	100
M2/M2′	MMGG GGMM	0	0
A/U′	CMXG GGCC	0	100
B/U′	CMGX GGCC	0	100
C/U′	CCXG GGCC	68	71
D/U′	CCGX GGCC	0	100
C/M′	CCXG GGMC	0	100
D/M′	CCGX GGMC	0	46

M = 5-methylcytosine X = 8-OH-deoxyguanosine

Hpa II is a methylation sensitive restriction endonuclease which recognizes 5′-CCGG-3′ in double-stranded DNA, but will not cleave the DNA if either or both of the cytosines is 5-methylcytosine; *Msp* I is an isoschizomer of *Hpa* II which will not cleave the sequence if the 5′ cytosine is methylated, but will cleave whether or not the 3′ cytosine is methylated. In addition, *Msp* I can cleave hemimethylated DNA, whereas *Hpa* II cleaves it poorly or not at all[24]. As can be seen in Table I, both enzymes exhibit expected activity with respect to native, hemimethylated, and fully methylated sequences (U/U′, U/M′, M/M′ and M2/M2′). The enzymes also demonstrate predicted activity with respect to methylation status in A/U′, B/U′, and C/M′. However, while *Msp* I cleaves D/U′, it has reduced ability to cleave C/U′ and D/M′; *Hpa* II does not cleave D/M′ (as expected based on methylation status) nor D/U′, and has reduced ability to cleave C/U′.

These results indicate that the presence of 8-OH-dG as the 3′ guanosine residue on one strand of the 5′-CCGG-3′ recognition site completely inhibits normal activity of the *Hpa* II endonuclease (D/U′), but exerts a lesser affect on the activity when in the 5′ guanosine position (C/U′). *Msp* I endonuclease activity is diminished when both the 3′ guanosine is 8-OH-dG and the 3′ cytosine of the complementary strand is 5-methylcytosine (D/M′), and to a lesser extent when the only modification is a 5′ 8-OH-dG (C/U′). *Msp* I activity is unaffected by the presence of a 5′ 8-OH-dG on one strand and a 3′ 5-methylcytosine on the complementary strand (C/M′).

The effect of 8-OH-dG on cleavage by these enzymes may be due to a decrease in the ability of the enzymes to recognize and bind to the recognition sequence, similar to the phenomenon we observed previously in studies examining the effect of 8-OH-dG on methylation by *Hpa* II methyltransferase[18]. Alternatively, the enzymes may bind to the recognition sequence but be unable to cleave the DNA. Further studies to determine the exact mechanism of cleavage inhibition are necessary.

That oxidative damage to DNA can produce alterations in the ability of restriction endonucleases to cleave DNA at their recognition sites is consistent with our earlier observations that such damage can affect enzymatic methylation of DNA[18]. DNA adduct formation via damage by reactive oxygen species may influence other DNA-protein interactions as well.

Acknowledgments

Supported by a generous grant from The Buehler Center on Aging and by National Institutes of Health Grant RO1 AG11536. We would also like to thank Steven S. Smith for his useful suggestions.

References

1. J.R. Wagner, C.-C. Hu and B.N. Ames (1992) Endogenous oxidative damage of deoxycytidine in DNA. *Proceedings of the National Academy of Sciences USA*, **89**, 3380–3384.
2. E. Schacter, E.J. Beecham, J.M. McCovey, K.W. Kohn and M. Potter (1988) Activated neutrophils induce prolonged DNA damage in neighboring cells. *Carcinogenesis*, **9**, 2297–2304.
3. B. Halliwell and O.I. Aruoma (1991) DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Letters*, **281**, 9–19.
4. S.A. Weitzman and L.I. Gordon (1990) Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. *Blood*, **76**, 655–663.
5. B. Ames (1991) Oxygen radicals and 8-hydroxyguanine in DNA. *Japanese Journal of Cancer Research*, **82**, 1460–1461.
6. R. Olinski, T. Zastawny, J. Budzbon, J. Skokowski, W. Zegarski and M. Dizdaroglu (1992) DNA base modifications in chromatin of human cancerous tissues. *FEBS Letters*, **309**, 193–198.
7. R. Cathcart, E. Schwiers, R.L. Saul and B.N. Ames (1984) Thymine glycol and thymidine glycol in

human and rat urine: a possible assay for oxidative DNA damage. *Proceedings of the National Academy of Sciences USA*, **81**, 5633–5637.
8. C. Richter, J.W. Park and B.N. Ames (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proceedings of the National Academy of Sciences USA*, **85**, 6465–6467.
9. R. Adelman, R.L. Saul and B.N. Ames (1988) Oxidative damage to DNA: relation to species metabolic rate and life span. *Proceedings of the National Academy of Sciences USA*, **85**, 2706–2708.
10. B. Halliwell and J.M.C. Gutteridge (1989) *Free Radicals in Biology and Medicine, 2nd Edition*. Clarendon Press, Oxford.
11. R.G. Cutler (1991) Antioxidants and aging. *American Journal of Clinical Nutrition*, **53**, 373S–379S.
12. B.N. Ames (1983) Dietary carcinogens and anticarcinogens. *Science*, **221**, 1256–1264.
13. B.N. Ames (1989) Endogenous oxidative DNA damage, aging, and cancer. *Free Radical Research Communications*, **7**, 121–128.
14. R.A. Floyd, J.J. Watson, J. Harris, M. West and P.K. Wong (1986) Formation of 8-hydroxydeoxyguanosine, hydroxyl free radical adduct of DNA in granulocytes exposed to the tumor promoter, tetradeconylphorbolacetate. *Biochemical and Biophysical Research Communications*, **137**, 841–846.
15. H. Kasai, Y. Okada, S. Nishimura, M.S. Rao and J.K. Reddy (1989) Formation of 8-hydroxydeoxyguanosine in liver DNA of rats following long-term exposure to a peroxisome proliferator. *Cancer Research*, **49**, 2603–2605.
16. C.G. Fraga, M.K. Shigenaga, J.W. Park, P. Degan and B.N. Ames (1990) Oxidative damage to DNA during aging: 8-hydroxy-2′-deoxyguanosine in rat organ DNA and urine. *Proceedings of the National Academy of Sciences USA*, **87**, 4533–4537.
17. D. Roy, R.A. Floyd and J.G. Liehr (1991) Elevated 8-hydroxydeoxyguanosine levels in DNA of diethylstilbestrol-treated Syrian hamsters: Covalent DNA damage by free radicals generated by redox cycling of diethylstilbestrol. *Cancer Research*, **51**, 3882–3885.
18. S.A. Weitzman, P.W. Turk, D.H. Milkowski and K. Kozlowski (1994) Free radical adducts induce alterations in DNA cytosine methylation. *Proceedings of the National Academy of Sciences USA*, **91**, 1261–1264.
19. D.C. Malins and R. Haimonot (1991) Major alterations in the nucleotide structure of DNA in cancer of the female breast. *Cancer Research*, **51**, 5430–5432.
20. W.A. Pryor (1988) Why is the hydroxyl radical the only radical that commonly adds to DNA? Hypothesis: it has a rare combination of high electrophilicity, high thermochemical reactivity, and a mode of production that can occur near DNA. *Free Radical Biology and Medicine*, **4**, 219–223.
21. M.L. Wood, M. Dizdaroglu, E. Gajewski and J.M. Essigmann (1990) Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. *Biochemistry*, **29**, 7024–7032.
22. D.S. Jones, F. Nemoto, Y. Kuchino, E. Ohtsuka and S. Nishimura (1990) 8-Hydroxydeoxyguanosine in DNA inhibits restriction endonuclease digestion. *Nucleosides and Nucleotides*, **9**, 223–233.
23. M. Moriya, C. Ou, V. Bodepudi, F. Johnson, M. Takeshita and A.P. Grollman (1991) Site-specific mutagenesis using a gapped duplex vector: a study of translesion synthesis past 8-oxodeoxygaunosine in *E. coli*. *Mutation Research*, **254**, 281–288.
24. M. McClelland, M. Nelson and E. Raschke (1994) Effect of site-specific modification on restriction endonucleases and DNA modification methyltransferases. *Nucleic Acids Research*, **22**, 3640–3659.

Accepted by Professor B. Halliwell