Arch. Pharm. Res. 13(3), 252-256

Oxidative Damage to Bacterial DNA and Evidence for Its Repair

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(Received May 31, 1990)

Abstract□Oxidative damage to DNA can be caused by excited oxygen species, which are produced by radiation or are by-products of aerobic metabolism. Endogenous levels of 8-hydroxy-2'-deoxyguanosine (8-OH-dG), an adduct that results from the damage of DNA caused by hydroxyl radical, have been detected in *E. coli* and *S. typhimurium*. Treatment of bacterial cells with various concentrations of hydrogen peroxide caused a moderate increase in the 8-OH-dG content. The enzymatic release of 8-OH-dG from asocorbate/Cu (II)-treated DNA was effected by an extract of *E. coli* cells. These results indicate that 8-OH-dG is formed *in vivo* in bacterial DNA through endogenous oxidative mechanisms and on treatment with an oxygen radical-producing agent and that it is repairable.

Key words□Oxygen radicals, DNA adduct, repair enzyme activity.

Damage to DNA induced by free radicals such as superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical (OH·) appears to play an important role in mutagenesis and carcinogenesis[1]. In living cells, free radicals are formed *in vivo* during aerobic metabolism or external agents such as ionizing radiations[2, 3]. Although cells have developed various enzymatic and nonenzymatic systems to control excited oxygen species[4], a certain fraction escapes the cellular defense and may cause permanent or transient damage to DNA. The critical targets that may be affected by excited oxygen in mutagenesis have not yet been identified with certainty.

Since free radicals produce a broad spectrum of DNA damage, biological studies have recently focused on the assessment of the potential mutagenic and carcinogenic lesions. Also biochemists have studied enzymes that recognize oxidative DNA damage. The inherent problem associated with detecting low levels of oxidatively damage bases in DNA has been resolved by the identification of an oxidilized DNA adduct 8-OH-dG which can be quantitated with a high degree of sensitivity and selectivity by electrochemical detection following separation of enzymatic hydrolyzates of DNA by a reversed phase high-performance liquid chromatography (HPLC)[5-7]. The hydroxyl radical seems to be involved in the hydroxylation of guanine base at C-8 position[8]. Using this sensitive and selective technique, 8-OH-dG has been shown to occur in DNA *in vivo* following exposure to oxygen radical-producing agents[9]. The presence of endogenous levels of 8-OH-dG in nuclear and mitochondrial DNA of rat liver[10], DNA of various tissues of rats[11], and DNA isolated from HeLa cells[9] have been reported. Since many mutagens, tumor promoters and carcinogens are known to generate oxygen radicals *in vivo* which is though to be relevant to mutagenesis[12], 8-OH-dG formation in DNA may be related to carcinogenesis.

The principal aim of these studies has been to estimate endogenous levels of 8-OH-dG in DNA from bacteria, and to examine whether 8-OH-dG is formed in cellular DNA when intact bacterial cells were treated with oxygen radical-producing agent. In the course of these studies we implicated that repair mechanism for the removal of 8-OH-dG lesions may exist in bacteria, and here we present evidence that an extract *E. coli* contains such an activity.

EXPERIMENTAL METHODS

Materials

Hydrogen peroxide (Sigma), asocorbic acid (Sigma) and cupric sulfate (Aldrich) were used without further purification. Calf thymus DNA was from Sigma. All enzymes came from either Sigma or Boehringer Mannheim. 8-OH-dG was synthesized with the Udenfriend hydroxylation system[13].

E. coli strain K12 and *S. typhimurium* strain LT2 were grown overnight at 37°C in VBC salts. A sample of 1g of wet cells collected by centrifugation was suspended in 80 m*l* of 0.1 M phosphate, pH 7.4, and was divided into four parts. One part was used as control without treatment and the other three were treated

with different concentrations of hydrogen peroxide. After 1 h incubation at 37°C, the bacteria were isolated by centrifugation at 10,000g for 20 min and lysed.

Isolation of DNA

DNA was isolated from bacteria as previously described[14] with scale-up. Residual RNA was destroyed by incubation at 37°C for 30 min with a mixture of RNase T1 (50 U/m*l*) and RNase A (100μg/m*l*) in 0.05 M Tris-HCl, pH 7.4 DNA concentration were estimated spectrophotometerically using 20 A_{260}U/mg of DNA.

Enzymatic hydrolysis of DNA

DNA samples (4-5 A_{260} U/200μ*l*) in 0.02 M sodium acetate, pH 4.8, were digested to nucleotides with 20 μg of nuclease P1 at 37°C for 30 min, and then treated with 1.3 units of *E. coli* alkaline phosphatase in 0.1 M Tris-HCl, pH 7.4, at 37°C for 1 h to liberate the corresponding nucleosides.

HPLC-EC

The HPLC consisted of a solvent delivery pump, injetor, UV detector and a reversed-phase Supelcosil C-18 column (particle size, 3μM, 15×4.6 cm). The electrochemical detection was accomplished by Bioanalytical System (West Lafayette, IN) LC-4B amperometric detector with a glassay-carbon electrode. All the conditions are the same as previously described[10].

Ascorbate/Cu (II)-treated DNA

Calf thymus DNA (2 mg/m*l*) was incubated in 0.01 M phosphate, pH 7.4, with freshly prepared ascorbate/Cu (II) (1 mM/0.1 mM) for 1 h at 37°C. The treated DNA was purified by precipitating it with 2 volumes of cold ethanol (−20°C) and used as a substrate.

Preparation of E. coli cell extract

E. coli strain K12 was grown overnight in 200 m*l* LB medium. A sample of the wet cells collected by centrifugation (1,000g, 10 min) was washed with 0.9% NaCl/1 mM dithiothreitol/10 mM Tris-HCl, pH 7.6, and pelleted and resuspended in the same buffer. After the cell suspensions were sonicated at 0°C for 2 min and centrifuged at 12,000g for 10 min, the protein content of this suspernatant was determined by Bradford method[15].

Assay of repair enzyme activity

Reaction mixtures consisting of 250 μ*l* extract (12 mg protein/m*l*), 10 μg ascorbate/Cu (II)-treated DNA, and NaCl to 25 mM were incubated at 37°C. The reactions were terminated by the addition of 3 m*l* ethanol. DNA and protein in each sample were pelleted by centrifugation at 10,000g for 10 min. The supernatants were dried under reduced pressure, resuspended in 300 μ*l* of 0.5% methanol (v/v) and microfiltered (0.25 μm filter). Samples were analyzed by HPLC-EC.

RESULTS AND DISCUSSION

Endogenous levels of 8-OH-dG in bacterial DNA

Typical chromatographic profiles of 8-OH-dG found in DNA of *E. coli* are shown in Fig. 1. 8-OH-dG has been readily detected at subpicomole levels by HPLC-EC. An enzymatic hydrolyzate of DNA following HPLC produced four prominent peaks absorbing at 260 nm which corresponded to the normal deoxynucleosides while 8-OH-dG produced detectable

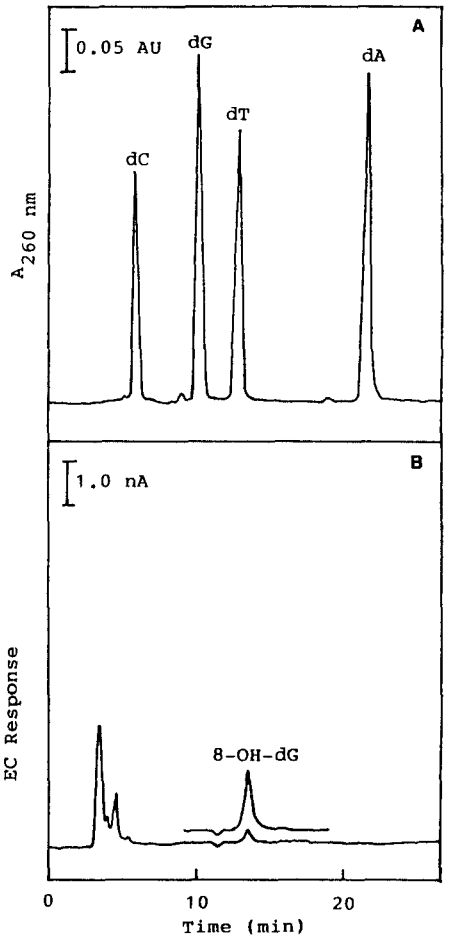

Fig. 1. HPLC chromatogram of an enzyme hydrolyzate of *E.coli* DNA analyzed by two dectection system linked in series.

A) HPLC chromatogram with UV detection at 260 nm. dC, deoxycytidine; dG, deoxyguanosine; dT, thymidine; dA, deoxyadenosine.

B) HPLC chromatogram with EC detection at +0.6 V. Inset: A partial EC chromatogram of the same sample spiked with 5 picomoles of authentic 8-OH-dG. Other details are described in the text.

Table I. Formation of 8-OH-dG in the DNA of bactreria exposed to hydrogen peroxide

Treatment (H_2O_2, mM)	8-OH-dG fmol/μg DNA	
	E. coli	*S. typhimurium*
0	3.2	3.8
0.06	3.7	4.9
1	8.2	9.3
2	9.8	10.1

signal at +0.6 V of electrochemical detector. endogenous levels of 8-OH-dG in DNA of *E. coli* and *S. typimurium* from both log and stationary phase cultures were measured. The results gave an average of 3.1 and 3.6 fmol 8-OH-dG/μg DNA for *E. coli* K12 and *S. typhimurium* LT2, respectively. This amount represents about 3-3.5 8-OH-dG residues/10^5 deoxyguanosine. These values are significantly lower than those of DNA from rat liver and HeLa cells (15-30 fmol 8-OH-dG/ μg DNA)[9, 10]. There was no appeciable difference between the log and stationary cultures. These results suggested that in normal conditions cellular DNA is damaged with formation of 8-OH-dG residues by oxygen radicals that are generated by normal cellular metabolism.

Treatment of bacteria with hydrogen peroxide

In the presence of transition metal catalyst the radiomimetic agent hydrogen peroxide (H_2O_2) is known to produce the highly reactive ultimate species, hydroxyl radical, responsible for the DNA damaging. As shown in Table I, only a moderate increase in the levels of 8-OH-dG was observed as the hydrogen peroxide concentration went up. This may indicate that the 8-OH-dG lesions are being repaired out of the DNA very efficiently.

Repair studies

Damage to DNA by oxygen free radicals has been documented to produce single strand breaks as well as modified thymidines[16, 17] and deoxyguanosine mainly at C-8 position[8]. Even though we cannot eliminate mutations resulting from several different types of damage, 8-OH-dG has been detected endogenously as a major oxidatively damaged DNA product. Furthermore, it was also demonstrated that an 8-OH-dG residue in synthetic oligonucleotides causes misincorporation during replication *in vitro* [18]. On the basis of the previous results about the 8-OH-dG levels in bacterial DNA we implicated that a repair mechanism for the potentially mutagenic 8-OH-dG lesions may exist in bacteria. Repair systems for the damage in thymidine lesions and single strand breaks have been already documented[19]. So far, no repair mechanism for 8-OH-dG has been described in the literature. Therefore, we focused on the detection of repair enzyme activity for the 8-OH-dG lesions from bacterial cells.

The simplest method for measuring repair involves determination of the loss of adduct in the DNA. it has often been technically difficult to observe the removal of a specific adduct from DNA[20]. Selectivity and sensitivity of HPLC-EC enable the detection of 8-OH-dG from repair of oxidatively damaged DNA. To determine whether bacterial cell extracts possess an 8-OH-dG repair enzyme activity, we used ascorbate/Cu(II)-treated DNA which contains 1.0-1.5 pmol 8-OH-dG/μg DNA as a substrate for assaying DNA repair enzyme activity.

HPLC-EC analysis of released 8-OH-dG from ascorbate/Cu(II)-treated DNA by the *E. coli* extrat is shown in Fig. 2. Confirmation of the identity of the released nucleoside was achieved by comparison of the HPLC elution profiles of its authentic standard and hydrodynamic voltammogram[6]. The rate of 8-OH-dG release *versus* protein concentration is plotted in Fig. 3 confirming that the excision of 8-OH-dG by the *E. coli* extract was probably enzymatic. Furthermore, when heat-denatured (100°C, 10 min) extract was used for the control, no excision of 8-OH-dG was observed. Either from the extract or ascorbate/Cu(II)-treated DNA alone, no 8-OH-dG was detecte even after 3 h incubation. The recovery of the spiked 8-OH-dG to the extract was higher than 95% , indicating that 8-OH-dG is not further metabolized in the cellular extract. This repair activity is probably some type of nucleotide excision repair which is the classical DNA excision scheme. Endonuclease activities acting on DNA irradiated with UV light or X-rays have been studied in calf thymus and rat liver. Although it has been proposed that some chemically altered residues might be subject to endonuclease activities, they did not identify the lesion recognized by the endonuclease[21,22].

The existence of such repair activity in itself suggests that 8-OH-dG residues in DNA are biologically undesirable to the cell. However, DNA repair is unlikely to remove all the lesions due to the continuously sustained oxidative stress, the total number of damage persisting in the DNA of an organism may be reflected as an endogenous level of 8-OH-dG. Recent interest in several laboratories has focused on potential global response to oxidative DNA damage, including those induced by oxidative stress. Although the induction of several proteins such as superoxide dismutase and catalase by a low dose of the pretreatment with prooxidants[23,24], as yet none of the indu-

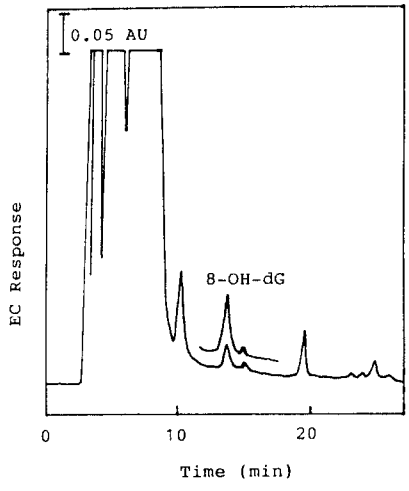

Fig. 2 HPLC-EC profiles of released 8-OH-dG from ascorbata/Cu(II)-treated DNA by the *E. coli* extract and the same sample spiked with authentic 8-OH-dG. Conditions are described in the text.

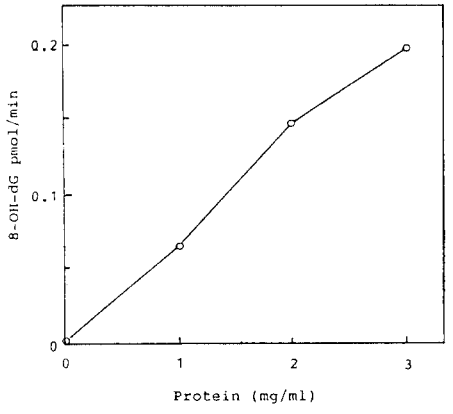

Fig. 3. *V Versus* $[E]_t$ plot of 8-OH-dG released by the *E. coli* extract.
Serial dilutions of extract were prepared and assayed for repair enzyme activity as described. The amount of released 8-OH-dG during each reaction was quantitated by HPLC-EC.

cible proteins has been shown to be a DNA repair enzyme.

The detoxification of reactive oxygen species is one of the prerequistes of aerobic life; among the multiple lines of defense possibly the biologically most important line of defense for the organsim consists in preserving the identity of the genetic material by repair after oxidative damage[4]. Therefore, DNA repair is considered as a universal and immediate response to DNA damage. At present, however, it is not clear that other types of repair enzymes for the 8-OH-dG lesions such as glycosylase also exist. This report presents results of demonstrating the maintenance of a low level of 8-OH-dG in bacteria probably, at least in part, by DNA repair.

ACKNOWLDGMENT

The author is deeply grateful to Dr. B.N. Ames for his support and valuable advice.

LITERATURE CITED

1. Cerutti, P.A.: Prooxidant states and tumor promotion. *Science* **227,** 375 (1985).
2. Ames, B.N.: Dietary carcinogens and anticarcinogens. *Science* **221,** 12556 (1983).
3. Teoule, R.: Radiation-induced DNA damage and its repair. *Int. J. Radiat. Biol.* **51,** 573 (1987).
4. Sies, H.: Biochemistry of oxidativae stress. *Angew. Chem. Int. Ed. Engl.* **25,** 1058 (1986).
5. Floyd, R.A., Watson, J.J., Wong, P.K., Altmiller, D.H. and Richard, R.C.: Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. *Free Radical Res. Commun.* **1,** 163 (1986).
6. Park, J.W., Cundy, K.C. and Ames, B.N.: Detection of DNA adducts by high-performance liqid chromatography with electrochemical detection. *Carcinogenesis* **10,** 827 (1989).
7. Shigenaga, M., Park, J.W., Cundy, K.C., Gimeno, C.J. and Ames, B.N.: Assaying *in vivo* oxidative DNA damage. *Methods Enzymol.* **186,** 522 (1990).
8. Kasai, H. and Nishimura, S.: Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res.* **12,** 2137 (1984).
9. Kasai, H., Crain, P.F., Kuchino, Y., Nishimura, S., Ootsuyama, A. and Tanoola, H.: Formation of 8-hydroxydeoxy-guanosine moiety in cellular DNA by agenst producing oxygen radicals and evidence for its repair. *Carcinogenesis* **7,** 1849 (1986).
10. Richter, C., Park, J.W. and Ames, B.N.: Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc. Natl. Acad. Sci. USA* **85,** 6465 (1988).
11. Fraga, C.G., Park, J.W. and Ames, B.N.: Oxidative damage to DNA evaluated by 8-hydroxydeoxyguanosine levels in DNA from organs of rats of different ages. *FASEB J.* **3,** 571 (1989), abstract.
12. Totter, J.R.: Spontaneous cancer and its possi-

ble relationship to oxygen metabolism. *Proc. Natl. Acad. Sci. USA* **77**, 1763 (1980).
13. Udenfriend, S., Clark, C.T., Axelrod, J. and Brodie, B.B.: Ascorbic acid in aromatic hydroxylation I. A model system for aromatic hydroxylation. *J. Biol. Chem.* **208**, 731 (1954).
14. Schleif, R.F. and Wisink, P.C.: *Practical methods in molecular biology.*, Springer-Verlag, New York, p.98 (1981).
15. Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248 (1976).
16. Kow, Y.W. and Wallace, S.S.: Exonuclease III recognizes urea residues in oxidized DNA. *Proc. Natl. Acad. Sci. USA* **82**, 8354 (1985).
17. Teebor, G.W., Frenkel, K. and Goldstenin, M. S.: Ionizing radiation and tritium transmutation both cause formation of 5-hydroxymethl-2'-deoxyuridine in celluar DNA. *Proc. Natl. Acad. Sci. USA* **81**, 318 (1984).
18. Kuchino, Y., Mori, F., Kasai, H., Inoue, H., Iwai, S., Miura, K., Ohtsuka, E. and Nishimura, S.: Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. *Nature* **327** 77 (1987).
19. Wallace, W.E.: AP endonucleases and DNA glycosylases that recognize oxidative DNA damage. *Environ. Mol. Mut.* **12**, 431 (1988).
20. Lindahl, T: DNA repair enzymes. *Annu. Rev. Biochem.* **51**, 61 (1982).
21. Tomura, T and van Lancker, J.L.: The effect of a mammalian repair endonuclease on X-irradiated DNA. *Biochem. Biophys. Acta* **402**, 343 (1975).
22. Bacchetti, S. and Benne, R.: Purification and characterization of an endonuclease from calf thymus acting on irradiated DNA. *Biochem. Biophys. Acta* **390**, 285 (1975).
23. Christman, M.F., Morgan, R.W., Jacobson, F.S. and Ames, B.N.: Positive control of a regulon for defense against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. *Cell* **41**, 753 (1985).
24. Morgan, R.W., Chraistman, M.F., Jacobson, F.S. and Ames, B.N.: Hydrogen peroxide-inductible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. *Proc. Natl. Aca. Sci. USA* **83**, 8059 (1968).

Note added in proof

During the preparation of this manuscript a report appeared presenting an identification of endonuclease activity in *E. coli* that repairs 8-OH-dG in DNA. [Chung *et al.*, 48th Meeting of the Japanese Cancer Association, October 1989 (Abstr. 198)]