Free Rad. Res. Comms., Vol. 2, No. 4–6, pp. 279–284
Photocopying permitted by license only
© 1987 Harwood Academic Publishers GmbH
Printed in Great Britain

THE ROLE OF HYDROXYL RADICALS IN THE DEGRADATION OF DNA BY OZONE

J. VAN DER ZEE, T.M.A.R. DUBBELMAN and J. VAN STEVENINCK†

Dept. Medical Biochemistry, Sylvius Laboratories, P.O. Box 9503, 2300 RA Leiden, The Netherlands

(Received July 21st 1986)

The degradation of the nucleotides dAMP, dGMP, dCMP and dTMP and of calf thymus DNA by ozone was studied. In all cases both base and sugar moiety were degraded. Furthermore, strand breaks were induced in calf thymus DNA. Hydroxyl radicals were probably involved in the oxidation of the base in dAMP and of the deoxyribose ring, but not in the degradation of the other bases. This indicates that ozone-induced DNA damage proceeds both directly via ozone molecules and indirectly via hydroxyl radicals.

KEY WORDS: Ozone, DNA, hydroxyl radicals, strand scission.

INTRODUCTION

Cell damage and tissue injury, initiated by free radicals has been given much attention lately, with special emphasis on the formation of the hydroxyl radical (OH^0).[1,2] This species reacts with various cellular constituents. Considerable evidence has been accumulated about the involvement of OH^0 in causing DNA base alteration and strand breaks in DNA. This type of damage has been shown to occur with radiation (e.g. X, α, β, and γ rays),[3] by an iron- or copper salt catalyzed Fenton type reaction, requiring both O_2^{0-} and H_2O_2[4] and by chemicals like bleomycin[5] and antracycline.[6]

Ozone, a very powerful oxidant, degrades under physiological conditions, yielding OH^0.[7] Hoigné and Bader[8] have shown that ozone-induced oxidations may occur either by a direct reaction between target molecules and ozone or via OH^0 as oxidizing intermediate. For example, the ozone-induced hydroxylation of phenylalanine and salicylic acid proceeds via OH^0.[9] Ozone also reacts with nucleic acids and derivatives,[10] but little is known about the mechanism by which ozone induces DNA damage. In the present paper the involvement of OH^0 in the degradation of the base and sugar moiety of several deoxyribonucleotides by ozone is studied, together with the formation of strand breaks in calf thymus DNA.

MATERIALS AND METHODS

dAMP, dGMP, dCMP, dTMP and calf thymus DNA type I were obtained from Sigma. All chemicals used were of analytical grade.

Ozone was generated by a Fisher Ozone-generator Model 501. Ozone production

† To whom correspondence should be addressed.

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/01/11
For personal use only.

RIGHTSLINK

was measured by titration with KI-$Na_2S_2O_3$-starch in 5 mM Na-phosphate (pH 7.4). 1 mM solutions of the deoxyribonucleotides in 50 mM Na-phosphate (pH 7.4) were exposed to an O_2/O_3 mixture containing 20 μmol O_3/ml. Calf thymus DNA was dissolved in 50 mM Na-phosphate (pH 7.4) at a concentration of 0.75 mg/ml. Base degradation was determined by the change in optical density at 260 nm. The formation of thiobarbituric acid (TBA) reactive material was assayed according to Gutteridge.[11] DNA strand breaks were assayed by alkaline sucrose gradient centrifugation. A 5–20% (w/v) linear sucrose density gradient was prepared as described before.[12] A 100 μl sample of ozone-treated DNA was added to 100 μl of 1 M NaOH and 10 mM EDTA and the mixture was layered on top of the gradient. The gradient was centrifuged for 5 hr at 40 000 rpm and 20°C. After centrifugation the gradient solution was fractionated and A_{260} was determined.

RESULTS

Exposure of dAMP, dGMP, dCMP and dTMP to ozone led to a decrease in A_{260} and to the formation of TBA-reactive material, as shown in Figure 1. Addition of 100 mM isopropanol or t-butanol had no effect on the degradation of the base moiety of dGMP, dCMP and dTMP, whereas it completely inhibited the degradation of the base moiety of dAMP. The formation of TBA-reactive material as a result of deoxyribose-breakdown by ozone, was completely inhibited in all cases upon addition of isopropanol or t-butanol.

Oxidation of hypoxanthine by ozone in the presence and absence of isopropanol is shown in Figure 2. Apparently isopropanol offered a partial protection against oxidation in this case.

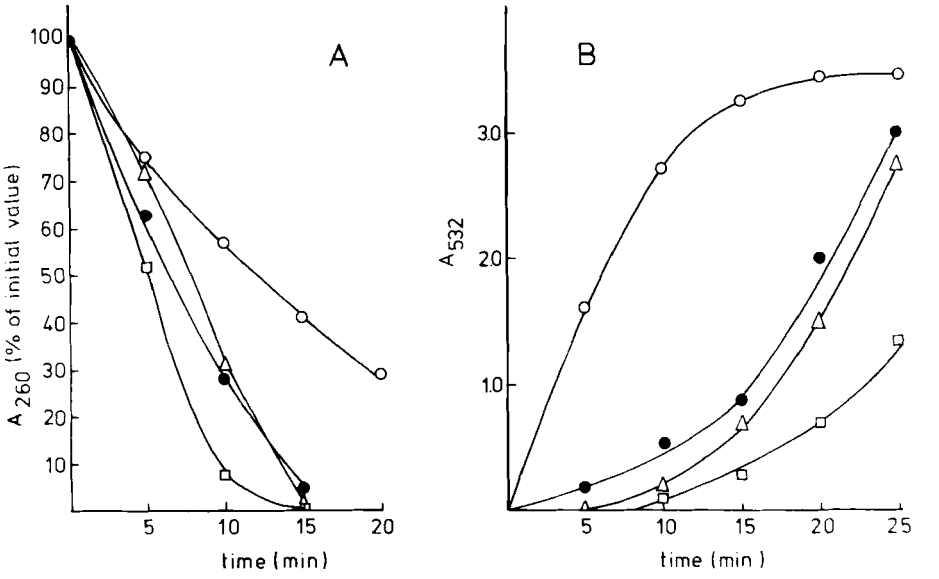

FIGURE 1 Degradation by ozone of dAMP (○), dGMP (●), dCMP (△) and dTMP (□). (A) decrease in A_{260}; (B) formation of TBA-reactive material (A_{532}).

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/01/11
For personal use only.

RIGHTSLINK

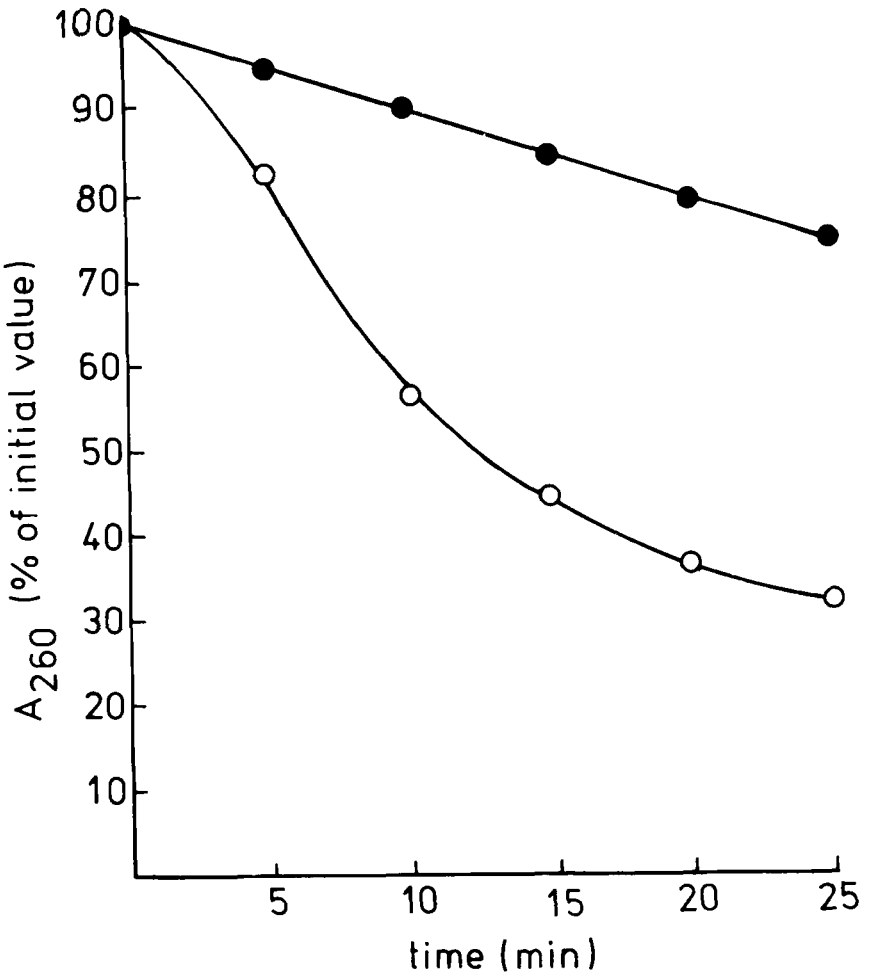

FIGURE 2 Degradation by ozone of hypoxanthine (shown as decrease in A_{260}), without (○) and with (●) 100 mM isopropanol.

Exposure of calf thymus DNA to ozone resulted in a decrease in A_{260} and in formation of TBA-reactive material (Figure 3). Addition of isopropanol or t-butanol completely inhibited the formation of TBA-reactive material, whereas the decrease in A_{260} was partly inhibited, isopropanol being more effective than t-butanol (Figure 3). Strand scission was detected using an alkaline sucrose gradient. Exposure of DNA to ozone led to a shift in the DNA peak pattern (Figure 4). Addition of isopropanol during ozone exposure had no effect upon this change, indicating that strand break formation was not inhibited by this OH^0 scavenger.

DISCUSSION

Ozone is an extremely reactive oxidant. It reacts with proteins, nucleic acids, unsaturated fatty acids and carbohydrates. As shown in Figure 1, all DNA base moieties

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/01/11
For personal use only.

RIGHTSLINK

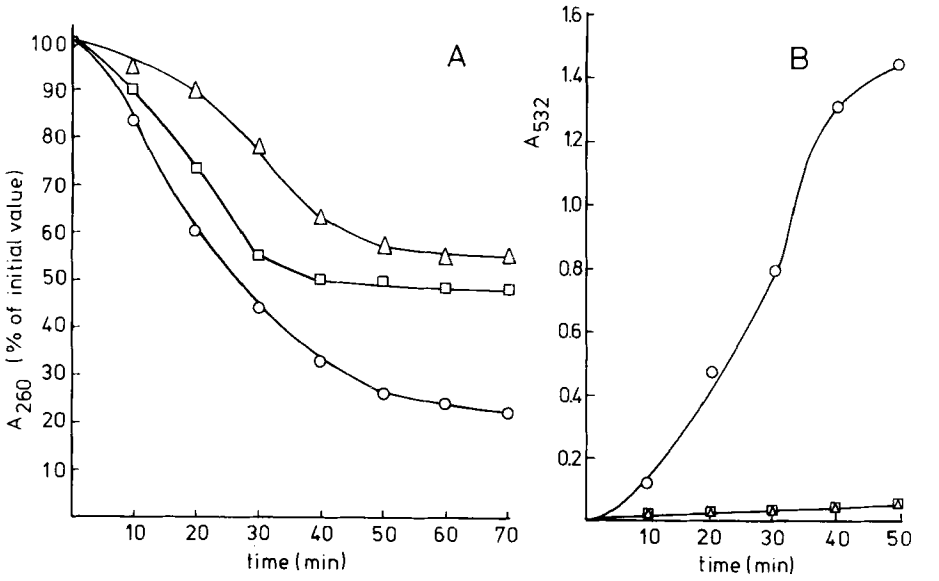

FIGURE 3 Degradation by ozone of calf thymus DNA, (○) no further additions; (△) 100 mM isopropanol added; (□) 100 mM t-butanol added. (A) decrease in A_{260}; (B) formation of TBA-reactive material (A_{532}).

were degraded by ozone. Addition of isopropanol or t-butanol only affected the degradation of adenine. Therefore OH^0 seems to be involved in the degradation of adenine, whereas guanine, cytosine and thymine are probably degraded by direct action of ozone molecules. Comparison of the structure of the four bases shows that only in the case of adenine the C-2 position is not occupied by a hydroxyl or amino group. Ozone-induced degradation of hypoxanthine, a purine with also a free C-2 position, is partly inhibited by isopropanol (Figure 2), indicating that oxidation occurs both directly by ozone, and indirectly, via OH^0. These results suggest, that a free C-2 position is required for OH^0-mediated oxidation.

Degradation of the deoxyribose ring can be detected by measuring the release of TBA-reactive material.[11] The sugar moiety was degraded by ozone. In the case of dCMP and dTMP, degradation of the deoxyribose ring started after complete oxidation of the pyrimidine ring. With dGMP and dAMP, both the sugar and the base moiety were oxidized simultaneously. The degradation of the deoxyribose ring was completely inhibited by isopropanol and t-butanol, indicating that degradation of the deoxyribose ring of all four deoxyribonucleotides proceeds via OH^0.

Exposure of calf thymus DNA to ozone also led to oxidation of both the base and the sugar moiety (Figure 3) and to strand scission (Figure 4). Experimental evidence presented by Giloni *et al.*[5] indicates that in bleomycin-induced DNA fragmentation presumably OH^0 are involved. These authors proposed a model in which OH^0-induced oxidation of the deoxyribose moiety leads to direct strand breaks and the generation of TBA-reactive degradation products. The fragmentation of DNA with the concomitant generation of TBA-reactive products, as shown in Figures 3 and 4, suggests a similar mechanism in ozone-exposed DNA. One observation, however, can

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/01/11
For personal use only.

RIGHTSLINK

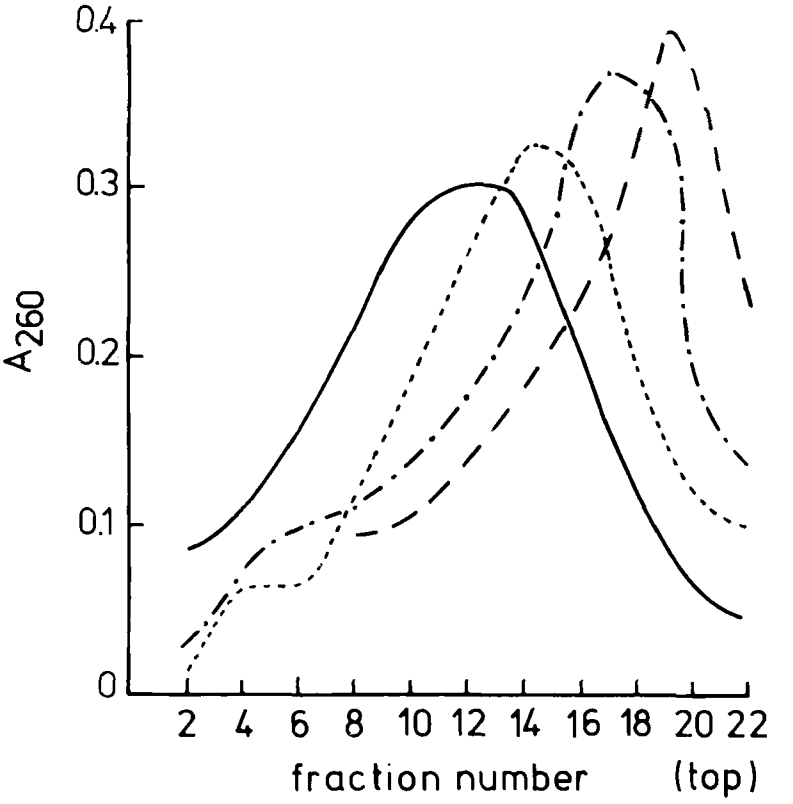

FIGURE 4 Density gradient centrifugation of calf thymus DNA, exposed to ozone. —— , 0 min; - - - - -, 5 min; –·–·–·–, 10 min; – – –, 15 min.

not be explained along these lines: the OH^0 scavenger isopropanol completely inhibited the generation of TBA-reactive products (Figure 3), without significant influence on strand scission (Figure 4). Possibly a direct attack of ozone leads to strand breaks without a concomitant formation of TBA-reactive products. Alternatively, base oxidation creates alkali-labile sites, disrupting during alkaline gradient centrifugation. In further experiments it will be tried to discriminate between these possibilities.

Very little data are available concerning the effects of ozone on cellular DNA. Mutational changes in *E. coli*[13] and *S. cerevisiae*,[14] aberration of chromosomes in human cells[15] and inhibition of DNA replication in Chinese hamster cells[16] have been shown. Further studies will have to decide whether in intact cells ozone-induced DNA damage is caused by similar mechanisms as observed in model systems.

Acknowledgements

The authors are grateful to Mr. A.R. Filon for his help with the gradient experiments. This work was supported by a grant of the Netherlands Organization for the Advancement of Pure Scientific Research, Z.W.O. (grant 98-61).

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/01/11
For personal use only.

RIGHTSLINK

References

1. Freeman, B.A. and Crapo, J.D. *Lab. Invest.*, **47,** 412 (1982).
2. Slater, T.F. *Biochem. J.*, **222,** 1 (1984).
3. Schulte-Frohlinde, D. and von Sonntag, C. in *Oxidative Stress*, ed. H. Sies (Academic Press: London, 1985) p. 11.
4. Mello Filho, A.C. and Meneghini, R. *Biochim. Biophys. Acta,* **781,** 56 (1984).
5. Giloni, L., Takeshita, M., Johnson, F., Iden, C. and Grollman, A.P. *J. Biol. Chem.*, **256,** 8608 (1981).
6. Mundi, J.R.F., Sinha, B.K., Gianni, L. and Myers, C.E. *FEBS Letters,* **172,** 226 (1984).
7. Grimes, H.D., Perkins, K.K. and Boss, W.F. *Plant Physiol.*, **72,** 1016 (1983).
8. Hoigné, J. and Bader, H. *Science,* **190,** 782 (1975).
9. Verwey, H., Christianse, K. and Van Steveninck, J. *Chemosphere,* **11,** 721 (1982).
10. Ishizaki, K., Shinriki, N. and Ueda, T. *Chem. Pharm. Bull.*, **32,** 3601 (1984).
11. Gutteridge, J.M.C. *Biochem. J.*, **224,** 761 (1984).
12. van Zeeland, A.A., Smith, C.A. and Hanawalt, P.C. *Mutation Res.*, **82,** 173 (1981).
13. Hamelin, C., Sarhan, F. and Chung, Y.S. *Biochem. Biophys. Res. Commun.*, **77,** 220 (1977).
14. Dubeau, H. and Chung, Y.S. *Mol. Gen. Genet.*, **197,** 120 (1984).
15. Guerrero, R.R., Rounds, D.E., Olson, R.S. and Hackney, J.D. *Environ. Res.*, **18,** 336 (1979).
16. Rasmussen, R.E. *J. Toxicol. Environ. Health,* **17,** 119 (1986).

Accepted by Prof. H. Sies

Free Radic Res Downloaded from informahealthcare.com by University of Illinois Chicago on 11/01/11
For personal use only.

RIGHTSLINK