OZONE-INDUCED DNA DEGRADATION IN DIFFERENT DNA POLYMERASE I MUTANTS OF ESCHERICHIA COLI K12

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SUMMARY: Cell survival and DNA degradation were measured in polA1, polA'107, resA1 and pol+ strains of Escherichia coli K12 after treatment with ozone. Results indicate that DNA polymerase I is involved in the repair of ozone-induced lesions to DNA. Unrepaired single-strand breaks are probably responsible for the increased cell killing and DNA degradation observed in these mutants after ozonation, although other types of lesions such as base damage and/or DNA-protein crosslinks may also be induced by this strong oxidant.

INTRODUCTION

Considerable data have been accumulated indicating that ozone is a radiomimetic agent (1). Nevertheless, the nature of the lesion or lesions in DNA giving rise to the lethal and mutagenic effects observed in E. coli after ozonation (2-4) remains largely unknown.

The purpose of this investigation was to test the hypothesis that the chief damage to DNA by ozone is in the form of strand scissions. This hypothesis was suggested mainly by the observation that radiosensitive lex mutants of E. coli, deficient in the repair of X-ray-induced single-strand breaks in DNA (5), are abnormally sensitive to ozone (6).

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DNA polymerase I plays a major role in the repair of radiation damage (5, 7); and extensive DNA degradation has often been found to correlate with sensitivity to irradiation and the lack of repair of DNA single-strand breaks (7, 8). Thus it seemed interesting to compare the DNA degradation response of different radiosensitive DNA polymerase I mutants after exposure to ozone.

MATERIALS AND METHODS

The following closely related strains of E. coli K12 were used: KMBL1787 (polA1), KMBL1788 (pol^+), KMBL1789 (polA'107), and KMBL1791 (resA1). The four strains were all F^-, thyA301, argA103, pheA97, bio-87, endA101. Cells were grown overnight at 37°C in glucose-salts medium (M9; ref. 9) supplemented with the necessary growth factors to the following concentrations: biotine, 0.2 µg/ml; thymine, 50 µg/ml; DL-amino acids, 40 µg/ml; L-amino acids, 20 µg/ml. For degradation experiments, bacterial DNA was labeled by incubation of the cells in the above medium containing 10 µCi of thymidine-^{3}H (specific activity: 40-60 Ci/mM) per ml, purchased from New England Nuclear. Grown cells were washed twice, resuspended in the same volume of fresh medium without label, and incubated with aeration at 37°C for another 1.5 h. After washing once more, they were suspended in M9 for ozonation. Undiluted cell suspensions (5 ml) were exposed to 50 ppm ozone for 30 min as described elsewhere (2). Following ozonation, 0.1 ml-samples were serially diluted, and spread on nutrient agar plates (Difco) to estimate the survival fraction (2). The procedure for measuring ozone-induced DNA degradation was that of Strike and Emmerson (10). Control experiments were carried out as above, except that clean air was used instead of ozone.

RESULTS AND DISCUSSION

It has been reported that the polA1 and resA1 strains are extremely X-ray sensitive while the polA'107 strain show an intermediate X-ray sensitivity when compared to the wild-type strain (11, 12); and, in accordance with radiation data, ozone surviving fractions of 8.9×10^{-4}, 1.1×10^{-3}, 1.8×10^{-2}, and 9.5×10^{-2} were obtained for strains KMBL1787, KMBL1791, KMBL1799, and KMBL1788, respectively. On the basis of these results, it thus appears that DNA polymerase I plays a key role in the repair of lesions produced in E. coli DNA by ozone; and that this strong oxidant probably

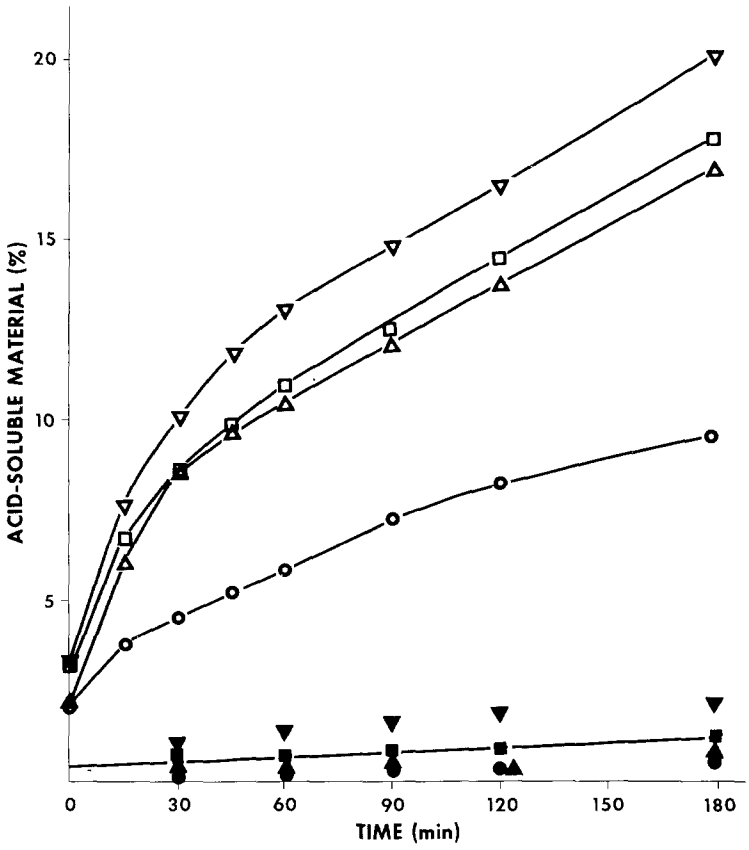

Figure 1. Kinetics of DNA degradation in E. coli K12 cells after a 30 min exposure to 50 ppm ozone. The extent of DNA degradation was measured as the fraction of labeled material soluble in 10% trichloroacetic acid. The results are the average of three independent experiments. ▽-▽, KMBL1787 (polA1); □-□, KMBL1789 (polA'107); △-△, KMBL1791 (resA1); O-O, KMBL1788 (pol^+). The corresponding control experiments are represented by a closed symbol.

affects the DNA in a similar fashion to ionizing radiation, notably by producing breaks in the polynucleotide chains.

To ascertain these possibilities, DNA degradation after ozonation was measured in these strains. From the results presented in Figure 1, it can be seen that ozone, as well as ionizing radiation, can induce extensive degradation of the DNA; and that the three

mutant strains degrade their DNA more extensively than wild-type strain after ozonation. Thus it seemed likely that unrepaired single-strand breaks in the DNA were responsible for the increased cell killing and DNA degradation observed in these phenotypically different mutants.

The fact that the polA1 and polA'107 strains degrade their DNA more rapidly and more extensively after ozonation than wild-type supports well the above conclusion since both mutant strains are partially deficient in single-strand break repair (11, 12). However, Gilckman et al. (11) have shown that although resA1 degrades more of its DNA than wild-type after X-irradiation, this mutant strain repairs X-ray single-strand breaks very efficiently. It thus seems possible that at least a part of the biological effects observed in E. coli after ozonation is due to lesions in DNA other than single polynucleotide chain breaks.

The reactivity of this strong oxidant towards nucleic acids and their derivatives (13-15), as well as proteins and amino acids (16-19), suggests that base damage (20) and/or DNA-protein crosslinks (21) may also be induced in DNA by ozone. Experiments are now in progress in this laboratory to determine the significance of such lesions in the sensitivity of E. coli to ozone.

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