JOURNAL OF BACTERIOLOGY, Jan. 1975, p. 392-395

Vol. 121, No. 1
Printed in U.S.A.

Rejoining of Radiation-Induced Single-Strand Breaks in Deoxyribonucleic Acid of *Escherichia coli*: Effect of Phenethyl Alcohol

C. K. K. NAIR, D. S. PRADHAN,* AND A. SREENIVASAN

Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay 85, India

Received for publication 9 August 1974

Single-strand breaks in deoxyribonucleic acid of *Escherichia coli* B/r cells exposed to 20 krads of gamma radiation could be rejoined by incubation of irradiated cells in growth medium. In the presence of 0.25% phenethyl alcohol, this repair was completely inhibited although deoxyribonucleic acid and protein syntheses were suppressed only partially.

Phenethyl alcohol (PEA) displays diverse effects on cellular processes in *Escherichia coli* in the bacteriostatic concentration range. It has been shown to inhibit a new round of deoxyribonucleic acid (DNA) replication (5), affect ribonucleic acid (RNA) and protein syntheses (8, 10), and inhibit formation of active alkaline phosphatase (12). In this communication, we present evidence to suggest that at the concentration of 0.25%, this chemical reversibly inhibits gamma ray-induced single-strand breaks in the DNA of *E. coli*.

DNA strand breaks were analyzed by the procedure of McGrath and Williams (7) as modified by Kaplan (4). *E. coli* B/r cells were grown in [^{3}H]thymidine to label DNA uniformly. The cells were irradiated in buffer. After irradiation they were incubated in growth medium in presence or absence of 0.25% PEA. At various times the cells were collected, converted to spheroplasts, lysed on the surface of alkaline sucrose gradients, and centrifuged (4). The sedimentation rate of DNA from cells exposed to 20 krads of gamma radiation (Fig. 1B) was much lower compared with that of DNA from unirradiated cells (Fig. 1A). Upon incubation of irradiated cells at 37 C for 1 h in growth medium, the sedimentation profile of cellular DNA displayed considerable shifts towards that of DNA from unirradiated cells (Fig. 1C). This indicates that almost all single-strand breaks introduced by radiation exposure were repaired. No such reversal of DNA sedimentation pattern of irradiated cells could be observable if the growth medium contained 0.25% PEA (Fig. 1D). Prolonging the incubation in the presence of this chemical for an additional 1 h also did not lead to restoration of the sedimentation pattern (Fig. 1E). PEA was ineffective in inhibiting elimination of single-strand breaks at concentrations lower than 0.2%. This chemical apparently does not introduce strand breaks in DNA by itself since incubation of unirradiated cells with 0.25% PEA for 2 h did not lower the DNA sedimentation rate (Fig. 1F). Single-strand breaks in DNA of irradiated cells that were previously incubated with PEA for 1 h were rejoined upon incubation in growth medium devoid of PEA for 1 h (Fig. 1G), indicating that the inhibitory effect of PEA on repair of DNA single-strand breaks is reversible.

PEA, at the concentration completely inhibiting repair of DNA single-strand breaks, allowed synthesis of DNA and protein to a certain extent (Fig. 2 and 3). This suggests that the action of PEA on DNA repair may not result from overall suppression of cellular metabolism. The partial inhibition of the synthesis of DNA and protein and complete blockage of single-strand break repair may be interrelated phenomena. RNA synthesis has been shown to be more sensitive to this agent than are protein and DNA syntheses (8, 10). On the basis of this observation, it has been postulated that PEA exerts its effects by primarily inhibiting RNA synthesis. In this context, it may be mentioned that the sensitivity of a new round of DNA replication to 0.25% PEA has been ascribed to specific inhibition of synthesis of a protein that is postulated to be involved in this process (5). A recent report indicates that protein synthesis may be required for rejoining of X ray-induced single-strand breaks in DNA (2). It is therefore quite plausible that PEA preferentially interferes with synthesis of protein(s) participating in DNA repair and thereby blocking this process.

Silver and Wendt (11) have shown that in the

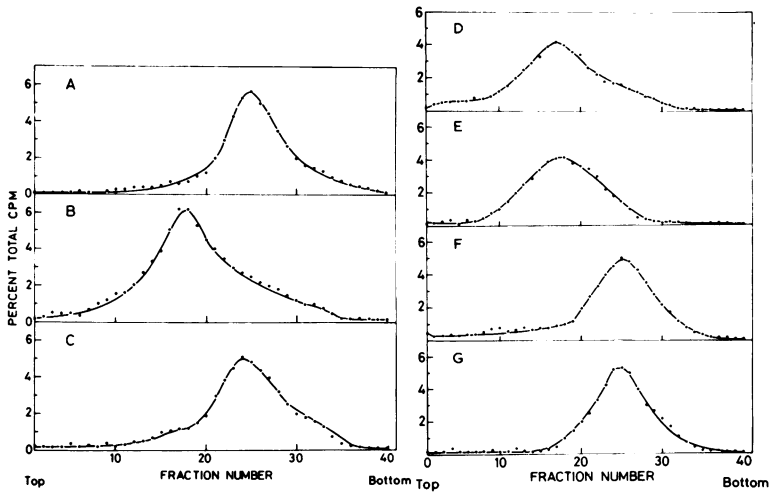

FIG. 1. *Sedimentation profiles of DNA from gamma-irradiated (20 krads) E. coli B/r cells after incubation in growth medium with or without 0.25% PEA. E. coli B/r was grown at 37 C for 17 h in M9 medium (6) supplemented with 0.4% glucose, 0.25% Casamino Acids, 50 μg of adenosine, (1) per ml and 100 μCi of [methyl-^{3}H]thymidine per ml (specific activity, 6 Ci/mmol). These cells were further incubated for 1 h in growth medium containing 100 μg of unlabeled thymidine per ml. The cells were suspended in 0.022 M phosphate buffer, pH 7.0, at a cell density of 10^8 cells/ml and exposed to 20 krads gamma rays under nitrogen. After irradiation, they were incubated in growth medium (at a density of 5×10^7 cells/ml) in presence or absence of 0.25% PEA for various times. Spheroplasts were prepared from these cells by the tris(hydroxymethyl)aminomethane-ethylenediaminetetraacetate-lysozyme procedure (4, 7). 0.17 ml of spheroplast suspension (5×10^6 spheroplasts) was overlayered on 0.18 ml of 1% dupanol placed on 4.8 ml of 5 to 20% linear sucrose gradient in 0.1 M NaOH and 0.9 M NaCl. The gradients were left standing for 15 min at room temperature and then centrifuged at 30,000 rpm for 40 min at 20 C by using an SW65 rotor in a Spinco L2-65B ultracentrifuge. After centrifugation, the gradients were fractionated by puncturing the tubes at the bottom and collecting 2-drop fractions on Whatman no. 3 filter paper disks (diameter, 2.4 cm). These disks were washed with trichloroacetic acid, alcohol, and acetone. After drying, the radioactivity in the disks was assayed by a Beckman LS-100 liquid scintillation spectrometer. The radioactivity of each fraction is expressed as percentage of the sum of radioactive counts in DNA in the gradient. The weight-average molecular weight of DNA (denatured in alkaline gradient) was calculated according to McGrath and Williams (7). (A) DNA from unirradiated cells, mass of 1.69×10^8 daltons; (B) DNA from 20-krad irradiated cells, 0-min incubation, mass of 1.14×10^8 daltons; (C) DNA from 20-krad irradiated cells, 1-h post-irradiation incubation in the absence of PEA, mass of 1.52×10^8 daltons; (D) DNA from 20-krad irradiated cells, 1-h incubation in the presence of 0.25% PEA, mass of 1.14×10^8 daltons; (E) DNA from 20-krad irradiated cells, 2-h incubation in the presence of 0.25% PEA, mass of 1.14×10^8 daltons; (F) DNA from unirradiated cells, 2-h incubation in the presence of 0.25% PEA, mass of 1.7×10^8 daltons; (G) DNA from 20-krad irradiated cells, 1-h incubation in presence of 0.25% PEA followed by 1-h incubation in absence of PEA, mass of 1.52×10^8 daltons.*

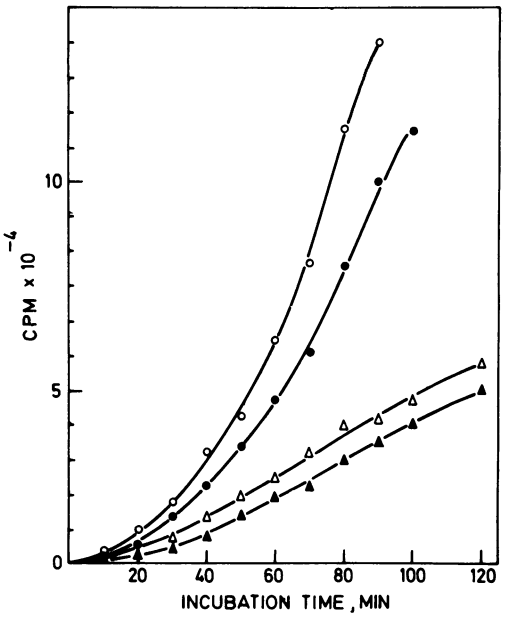

FIG. 2. *Incorporation of [^3H]thymidine into DNA in E. coli B/r cells during incubation in growth medium in the presence and absence of 0.25% PEA. After irradiation, E. coli B/r cells were incubated at 37 C in growth medium containing 10 μCi of [methyl-^3H]thymidine per ml (specific activity, 6 Ci/mmol) and 50 μg of adenosine per ml at a density of 5 × 10^7 cells/ml in the presence and absence of 0.25% PEA. At various times, 0.5-ml portions were transferred to 1 ml of cold 10% trichloroacetic acid and acid-insoluble radioactivity was determined. Symbols: ○, unirradiated cells incubated in the absence of PEA; ●, 20-krad irradiated cells incubated in the absence of PEA; △, unirradiated cells incubated in the presence of 0.25% PEA; ▲, 20-krad irradiated cells incubated in the presence of 0.25% PEA.*

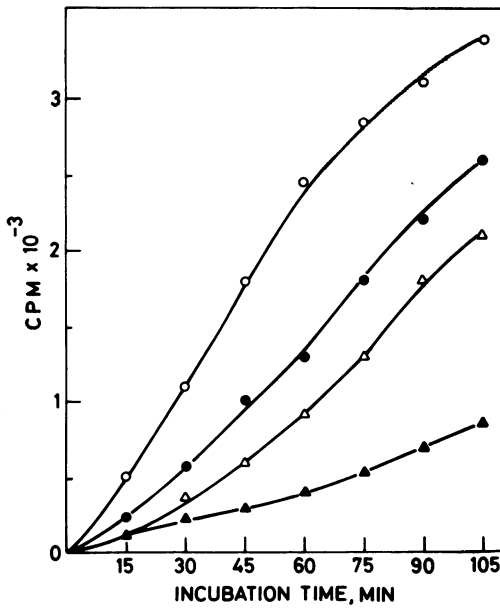

FIG. 3. *Incorporation of [^{14}C]leucine into proteins in E. coli B/r cells during incubation in growth medium in the presence and absence of 0.25% PEA. E. coli B/r cells, after irradiation, were incubated at 37 C at a density of 5 × 10^7 cells/ml in growth medium containing 10 μCi of [1-^{14}C]leucine (specific activity, 54 mCi/mmol) in the presence and absence of 0.25 of PEA. At various intervals, 0.5-ml portions were transferred to 1 ml of cold 10% trichloracetic acid and acid-insoluble radioactivity was determined. The curves are designated as in Fig. 2.*

presence of 0.25% PEA, there results an increased rate of efflux of cellular potassium in *E. coli*. Such leaking out of essential metal ions and/or small molecules may selectively impair one or more steps of DNA repair.

The changes in cell permeability brought about in the presence of PEA also indicate structural alterations in the cell membrane. Presumably, therefore, some of the effects of PEA may be elicited by structural changes induced by this agent in the cell membrane. There have been a number of reports in recent years indicating that in bacterial cells, DNA is attached at many points to the cell membrane (3, 9). If attachment of the cell membrane to DNA or integrity of the cell membrane is essential for DNA single-strand break repair, this process is likely to be susceptible to agents affecting the cell membrane. This hypothesis could be tested by using various known membrane-affecting agents.

Finally, the possibility that the inhibitory effect on the DNA repair could arise from specific sensitivity to PEA of one or more enzymes participating in the DNA repair also exists.

LITERATURE CITED

1. Doskocil, J., and V. Paces. 1968. The effect of nucleosides on the phosphorolysis of thymidine by normal and phage infected cells of *E. coli*. Biochem. Biophys. Res. Commun. **30**:153–158.
2. Ganesan, A. K., and K. C. Smith. 1972. Requirement for protein synthesis in *rec*-dependent repair of DNA in *Escherichia coli* K-12 after ultraviolet or X irradiation. J. Bacteriol. **111**:575–585.
3. Ivarie, R. D., and J. J. Pene. 1973. Association of many regions of the *Bacillus subtilis* chromosome with cell membrane. J. Bacteriol. **114**:571–576.
4. Kaplan, H. S. 1966. DNA strand scission and loss of viability after X-irradiation of normal and sensitized bacterial cells. Proc. Nat. Acad. Sci. U.S.A. **55**:1442–1446.
5. Lark, K. G., and C. Lark. 1966. Regulation of chromosome replication in *Escherichia coli*: a comparison of the effects of phenethyl alcohol treatment with those of amino acid starvation. J. Mol. Biol. **20**:9–19.

6. Lark, K. G., T. Repko, and E. J. Hoffman. 1963. The effect of amino acid deprivation on subsequent deoxyribonucleic acid replication. Biochim. Biophys. Acta **76**:9–24.
7. McGrath, R. A., and R. W. Williams. 1966. Reconstruction *in vivo* of irradiated *Escherichia coli* deoxyribonucleic acid; rejoining of broken pieces. Nature (London) **212**:534–535.
8. Prevost, C., and V. Moses. 1966. Action of phenethyl alcohol on the synthesis of macromolecules in *Escherichia coli*. J. Bacteriol. **91**:1446–1452.
9. Rosenberg, B. H., and L. F. Cavalieri. 1968. Shear sensitivity of the *E. coli* genome: multiple membrane attachment points of the E. coli DNA. Cold Spring Harbor Symp. Quant. Biol. **33**:65–72.
10. Rosenkranz, H. S., H. S. Carr, and H. M. Rose. 1965. Phenethyl alcohol. I. Effect on macromolecular synthesis of *Escherichia coli*. J. Bacteriol. **89**:1354–1369.
11. Silver, S., and L. Wendt. 1967. Mechanism of action of phenethyl alcohol: breakdown of the cellular permeability barrier. J. Bacteriol. **93**:560–566.
12. Tribhuvan, R. C., A. K. Pilgaokar, D. S. Pradhan, and A. Sreenivasan. 1970. Effect of phenethyl alcohol on induction of alkaline phosphatase in *Escherichia coli*. Biochem. Biophys. Res. Commun. **41**:244–250.