NATURE OF THE REPAIR PROCESS ASSOCIATED WITH THE RECOVERY OF ESCHERICHIA COLI AFTER EXPOSURE TO Cd^{2+}

R. S. Mitra and I. A. Bernstein
Cellular Chemistry Laboratory, Department of Environmental and Industrial Health and Department of Biological Chemistry,
The University of Michigan, Ann Arbor, Michigan 48109

Received November 22,1976

SUMMARY: When different strains of Escherichia coli are exposed to Cd^{2+}, the cells accommodate after a long lag and proliferate. The time required for this response depends on the nature of the strain and the supplements in the growth medium. Immediately after exposure to Cd^{2+}, considerable single strand breaks in the DNA are observed but the DNA is repaired prior to the initiation of cell proliferation. The finding that accommodation occurs in DNA polymerase I-deficient mutant cells suggests that DNA polymerase I may not be required for repair of damaged DNA in Cd^{2+}-exposed cells. The recovery of Cd^{2+}-exposed cells in a temperature-sensitive DNA ligase mutant cells at the permissive temperature (30° C) and failure to recover at the non-permissive temperature (42° C) indicates, however, that DNA ligase is involved in the repair of the single strand breaks associated with Cd^{2+}-induced damage.

INTRODUCTION

Escherichia coli strain B, growing in a synthetic medium, exhibit an abnormally long lag in the presence of 3×10^{-6} M Cd^{2+}. However, the cells eventually begin to proliferate at a normal rate and to a normal extent (henceforth these cells will be referred to as accommodated cells) (1). Immediately after exposure to Cd^{2+}, 85-95% of the cells lose their ability to form colonies on nutrient agar plates. This loss of viability is accompanied by considerable single (but no double) strand breakage in the DNA (2). The accommodative response includes an increase in the number of viable cells without a significant incorporation of [^{3}H]thymidine into DNA, no change in the turbidity of the culture and insensitivity to low concentrations of hydroxyurea suggesting that accommodation involves repair of damaged DNA. In this communication we report the possible role of DNA ligase in the repair of DNA although the mechanism of single strand breaks after Cd^{2+}-exposure is not clear.

ISSN 0006-291X

Table 1

Growth of *E. coli* in the presence of Cd^{2+}

E. coli strain	thymine		leucine, methionine, biotin and thymine	
	Time required to attain mid point of growth phase		Time required to attain mid point of growth phase	
	Control (hrs)	Cd^{2+} (hrs)	Control (hrs)	Cd^{2+} (hrs)
Strain B	5	20	5	13
JG 139 (pol^+)	8	25	8	13
JG 138 (pol^-)	7	25	9	26
DY 194 (temperature-sensitive DNA ligase defective cells)			12	36

The different strains used in this experiment have been indicated in the Table. Cells were inoculated to glucose-salts medium supplemented with 10 µg thymine, 10 µg L-leucine, 10 µg L-methionine and 2 µg biotin per ml of medium; 3×10^{-6} M Cd^{2+} was added at the time of cell inoculation. The culture was incubated at 37° C except the strain DY 194 which was incubated at 30° C. Growth was monitored with a Coleman Junior Spectrophotometer at 450 nm.

METHODS

Bacterial strains and growth conditions. *Escherichia coli* strain B was cultured aerobically in a glucose-salts synthetic medium at 37° C. The methods for the determination of cell viability and the turbidity of the cultures have been described previously (1). The other strains used in this work were JG 138 (F^- *rha lacZ str thyA thyR polA1*) and JG 139 (F^- *rha lacZ str thyA thyR*). These strains were grown in a glucose-salts medium supplemented with 10 µg of thymine per ml of medium. A temperature-sensitive, DNA ligase-defective strain, DY 194 (F^- *leuB rha lacZ str metE malB ligts-7 bio thyR*) was cultured aerobically in a glucose-salts medium with the supplements of 10 µg L-leucine, 10 µg L-methionine and 2 µg biotin per ml of medium. The strains JG 138, JG 139 and DY 194 were derived from K-12 and were obtained from Doctor D. A. Youngs, Stanford University.

Treatment of cells with Cd^{2+} and post-treatment conditions. Cells taken from a culture which had been growing for 16 hrs were inoculated into

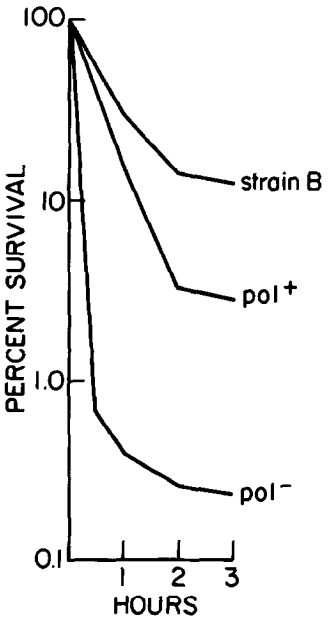

Fig. 1. Relative Cd^{2+}-sensitivities of selected strains of E. coli. Cells (approximate number 1.2 - 1.7 x 10^7/ml) in stationary phase were exposed to 3 x 10^{-6} M Cd^{2+} in glucose-salts medium containing 10 µg thymine per ml of medium. The culture was incubated at 37° C. The strains used in this experiment were strain B, JG 138 (pol^-) and JG 139 (pol^+).

---

the growth medium contaiing 3 x 10^{-6} M Cd^{2+} and were grown on a shaker. To study the effect of post-incubation in Cd^{2+}-free medium, cells after exposure to Cd^{2+} for 3 hrs, were harvested at room temperature, washed twice with medium and inoculated into fresh growth medium. Samples were then removed at appropriate times to determine the number of viable cells.

RESULTS AND DISCUSSION

When growing cultures of Escherichia coli were exposed to 3 x 10^{-6} M Cd^{2+}, the cells were found to accommodate to this toxic ion after a prolonged lag. The extent of the lag period in the presence of Cd^{2+} was found to greatly depend on the particular strain and the supplements in the medium. As shown in Table 1, the wild type strain (strain B and JG 139) had the ability to accommodate much more quickly when exposed to Cd^{2+} in the presence of added L-leucine, L-methionine, biotin and thymine while the mutant strains JG 138 and DY 194 did not. Further studies with E. coli

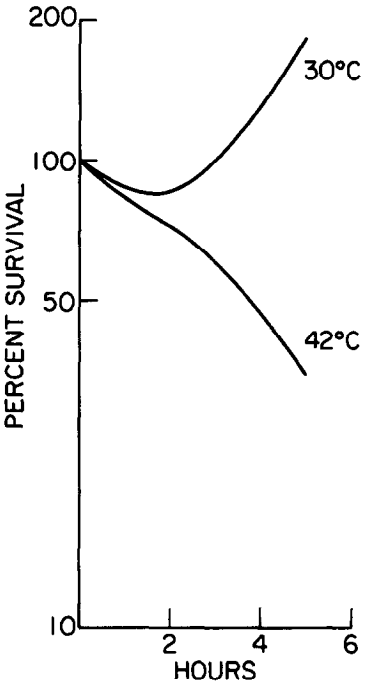

Fig. 2. Recovery of temperature-sensitive DNA ligase defective cells from the effect of Cd^{2+}. Growing cells (3×10^7/ml) were inoculated into 3×10^{-6} M Cd^{2+}-containing medium with the appropriate supplements and incubated at 30° C for 3 hrs. After 3 hrs of exposure, the cell viability of the culture was 1.5×10^6/ml. The bacteria were then harvested, washed twice with medium and resuspended in a small volume of growth medium. The cultures were divided into two equal portions and were inoculated into fresh growth medium. The cultures were incubated aerobically at 30° C or 42° C and the viable cell count was determined at intervals.

strain B or JG 139 indicated that leucine and methionine alone were effective in protecting the cell from Cd^{2+}-injury. The mechanism of this protective effect is currently under investigation.

The exposure of cells to Cd^{2+} in vivo resulted in single strand breaks, although in vitro exposure neither produced any strand breakage nor was the Cd^{2+} found to be associated with the DNA (2). Normally strand breaks may be repaired directly by DNA ligase; however, if nuclease activity converts a strand break into a strand gap, DNA synthesis would be required for

repair (3). It is believed that DNA polymerase I is involved in the excision and resynthesis steps of repair (4) and that the process is completed when apposed free ends are joined by DNA ligase (5). It has been shown that DNA polymerase I is a Zn-metalloenzyme and that the replacement of Zn^{2+} by Cd^{2+} leads to the complete inactivation of the enzyme (6). The displacement of Zn^{2+} from the enzyme as a result of exposure of cells to Cd^{2+}, might therefore, lead to the accumulation of degraded DNA. Studies with the pol A mutant cells (lacking DNA polymerase I) showed that this strain is more sensitive to Cd^{2+} than is the wild type strain. However, these mutant cells were also found to accommodate to Cd^{2+} (Table 1). Either a) the greatly reduced level of DNA polymerase I present in this strain (7) is sufficient to carry out the repair function, or b) another DNA polymerase, e.g. DNA polymerase III, plays a role in the repair of DNA (8) when DNA polymerase I is at a low level or c) no polymerase is needed.

It is possible that ligation of breaks is all that is necessary for repair of the Cd^{2+}-induced lesion. To study the involvement of DNA ligase in the postulated DNA repair phase of accommodative response, a mutant strain of E. coli having temperature-sensitive DNA ligase was used. These cells were found to accommodate to 3×10^{-6} M Cd^{2+} at the permissive temperature (30° C) after a long lag. The recovery process in these strains was studied by incubating the cells at 30° C and 42° C in Cd^{2+}-free medium after exposure to Cd^{2+} for 3 hrs. The results indicate (Fig. 2) that at the permissive temperature, the number of viable cells gradually increased whereas at the non-permissive temperature the number of viable cells gradually decreased. This observation confirms, as expected, that DNA ligase is necessary for repair of the single strand breaks in DNA and that this repair of DNA is essential for restoration of viability. If DNA ligase is indeed affected by Cd^{2+}, then during repair, the enzyme must be insensitive to Cd^{2+} in the cell or must be protected from the ion by some compartmentation of the ion or the enzyme.

There appears to be a difference in the sensitivity towards Cd^{2+} between the strains B and K-12 (Fig. 1). The exact reason for such difference is not known although similar differences between the strains B and K-12 in response to UV and ionizing radiations can be found in the literature (9).

ACKNOWLEDGMENTS

This investigation was partially supported by U.S. Public Health Service grant number 2 T01 AM 05268 and grant number 5 R01 AM 15206 from the National Institute of Arthritis, Metabolism and Digestive Diseases and by Institutional grant RR 05447, National Institutes of Health. The authors would like to thank Doctor D. A. Youngs for providing us with E. coli JG 138, JG 139 and DY 194.

REFERENCES

1. Mitra, R.S., Gray, R.H., Chin, B. and Bernstein, I.A. (1975) J. Bacteriol., 121, 1180-1188.
2. Mitra, R.S. and Bernstein, I.A. (1976) J. Bacteriol., submitted for publication.
3. Pauling, C., Beck, L.A. and Wilczynski, S.P. (1976) J. Gen. Microbiol. 94, 297-304.
4. Kelly, R.B., Atkinson, M.R., Huberman, J.A. and Kornberg, A. (1969) Nature, 224, 495-501.
5. Weiss, B. and Richardson, C.C. (1967) Proc. Nat. Acad. Sci., U.S.A. 57, 1021-1028.
6. Springgate, C.F., Mildvan, A.S., Abramson, R., Engle, J.L. and Loeb, L.A. (1973) J. Biol. Chem., 248, 5987-5993.
7. Olivera, B.M. and Bonhoeffer, F. (1974) Nature, 250, 513-514.
8. Hamelin, C., Youngs, D.A. and Smith, K.C. (1976) J. Bacteriol., 127, 1307-1314.
9. Sedgwick, S.G. (1975) J. Bacteriol., 123, 154-161.