The Effect of Colicin E2 on Bacteriophage ϕ X 174-Infected Cells of *Escherichia coli*

Akiko SETO,*¹ Takao SHINOZAWA,
Akio MAEDA and Tohru KOMANO*²

Department of Biophysic, Faculty of Sciences and

*²Laboratory of Biochemistry, Department of
Agricultural Chemistry,
Kyoto University, Kyoto

Received June 21, 1974

Degradation of DNA is the most remarkable phenomenon observed in colicin E2-challenged cells of *Escherichia coli*.¹⁾ Some kinds of treatment which may modify the physiological states of bacteria, however, affect on the extent of DNA degradation induced by E2.^{1~5)} It has also been reported that the extent of DNA degradation is reduced without the appreciable decrease of bacteriocidal effects in some kinds of strains^{6,7)} or in challenging a modified colicin.⁸⁾ In the course of studies to find the effects of colicin E2 on the replication of bacteriophage ϕ X 174, it was revealed that the infection of ϕ X 174 resulted in the depression of the action of colicin E2.

Figure 1 shows the effect of ϕ X 174 infection on the solubilization of *E. coli* HF 4704 DNA. The degradation of DNA into cold acid-soluble form became significant about 10 min after the addition of E2. Infection of ϕ X 174 on the cells challenged with E2 further retarded the start of the solubilization of host DNA and also somewhat reduced the rate of it. When ϕ X 174 was added at 30 min after the solubilization of DNA had started, the rate of it was also reduced. Chloramphenicol (100 μ g/ml) does not exert any effect on E2-induced solubilization of DNA in ϕ X-infected cells as is observed in uninfected cells.¹⁾ These results seemed to indicate that the function of E2 was affected by an early function of ϕ X 174 infection. It is reported that the expression of cistron A function proceeds even in the presence of 30 μ g/ml of chloramphenicol.¹¹⁾ The use of ϕ X 174 am33 (cistron A defective; kindly supplied by Dr. Sinshimer)¹²⁾ afforded the identical results with those from wild-type phage. Rifampicin (200 μ g/ml) also did not influence on the E2-induced solubilization in ϕ X-infected cells, while this drug somewhat enhanced the extent of solubilization in uninfected cells. A phage preparation inactivated to 10^{-5} from its original titers UV-irradiation also exerted the suppressive effect

*¹ Present Address: Institute of Life Science, Minami-oya, Machida, Tokyo.

FIG. 1. Effect of ϕ X 174 Infection on the Solubilization of DNA by Colicin E2.

E. coli HF4704 (*hcr*⁻, *thy*⁻; a derivative of *E. coli* C)⁹⁾ was grown in TPG-medium¹⁰⁾ containing 0.5 μ Ci/ml of ³H-thymidine and 2 μ g/ml of thymine to a density of 4×10^8 /ml. Cells were washed and suspended in TPG-medium containing 2 μ g/ml of thymine. The cell suspension was distributed into test tubes and shaken at 37°C. E2 (moi 60) and ϕ X 174 (moi 7) were added at 7 min and 12 min, respectively. Samples were taken out at intervals and poured into ice-cold 5% TCA. Precipitates were collected on glassfiber filters. Radioactivity was counted in a Packed Tricarb Liquid Scintillation Counter. Open symbols denote the results from uninfected cells and closed symbols from ϕ X 174-infected cells. Circles denote the results from the experiments without E2 and triangles from those with E2.

on the E2-induced DNA solubilization. Thus, the suppressive effect of ϕ X 174 infection on E2 action does not require the expression of gene-function of ϕ X 174. It has also been reported that the depression of E2 action is observable in the cells infected with bacteriophage T4 in the presence of chloramphenicol.^{1,5)}

No appreciable breakdown of parental and progeny ϕ X 174 DNA by E2 was noticed when E2 was added at the time of ϕ X 174 infection. No significant decrease on the number of produced phage particles was observed accompanying with E2-challenge (data not shown). Contrary to E2, colicin E3 (provided from Dr. Ohsumi) reduces appreciably the progeny phage production in the ϕ X 174-infected cells. Colicin E3 and colicin K stop the protein synthesis in ϕ X 174-infected cells in almost the same extent as in uninfected cells. It can be deduced that adsorption of ϕ X 174 particles alters the cells to be resistant to E2, but not to E3 and K.

Some other effects of E2 were noticed in these phage-

FIG. 2. Changes in the Turbidity of E2-Treated Culture.

E. coli HF4704 was grown in TPG medium to a cell density of 2×10^8 /ml, cooled and distributed into four Thunberg-type cuvettes. E2 (moi 30) and ϕ X 174 *am3* (moi 4) were added. All the cuvettes were shaken at 37°C. Optical density at 550nm was measured at intervals. Open symbols denote the results from uninfected cells and closed symbols from ϕ X 174-infected cells. Circles denote the results from the experiments without E2 and triangles from those with E2.

infected cells when the change in the turbidity of culture was followed (Fig. 2). As the phage used here is ϕ X 174 *am3* being lysis-defective, infection of ϕ X 174 did not result in the decrease of turbidity in the infected cells in the absence of E2. When E2 was added to uninfected culture the increase of turbidity ceased after about one generation time and the turbidity thereafter remained at a constant level. The addition of E2 to ϕ X-infected culture revealed a different feature. The rate of turbidity increase became slower than that of

other cultures after passing about 20 min and the turbidity rather fell down with further duration of incubation. Phage titers liberated in the medium at 180 min were determined after the addition of 1 mM EDTA to withdraw reversibly adsorbed phage particles from receptor sites followed by spinning down of cells. The number of titers in the supernatant fraction comprised only 1% of the total number of particles accumulated inside cells at this time. It is supposed that E2-challenged cells have not suffered from lysis to expel the cellular contents even in a situation where turbidity decreases considerably. It has been known that the ghosts of T4 modify the cellular membrane.¹⁴⁾ Although the nature of the turbidity depression is remained still open, it would be expected that the ϕ X 174-stuck membrane is changed so as to modify the function of colicin E2.

REFERENCES

- 1) M. Nomura, *Cold Spring Harbor Symposia on Quant. Biol.*, **28**, 315 (1963).
- 2) T. Beppu and K. Arima, *J. Bacteriol.*, **93**, 80(1967).
- 3) T. Beppu and K. Arima, *J. Biochem. (Tokyo)*, **70**, 263 (1971).
- 4) E. M. Holland and I. B. Holland, *Biochim. Biophys. Acta*, **281**, 179 (1972).
- 5) R. L. Swift and J. B. Wiberg, *J. Virol.*, **8**, 303 (1971).
- 6) K. Nose and D. Mizuno, *Biochim. Biophys. Acta*, **246**, 20 (1971).
- 7) T. Beppu, K. Kawabata and K. Arima, *J. Bacteriol.*, **110**, 485 (1972).
- 8) C. Kennedy and E. Sodergren, *J. Bacteriol.*, **112**, 736 (1972).
- 9) B. H. Lindgqvist and R. L. Sinsheimer, *J. Mol. Biol.*, **28**, 87 (1966).
- 10) R. L. Sinsheimer, B. Starman, C. Nagler and S. Guthrie, *J. Mol. Biol.*, **4**, 142 (1962).
- 11) A. Levine and R. L. Sinsheimer, *ibid.*, **32**, 567 (1968).
- 12) R. L. Sinsheimer, *Progress in Nucleic Acid Research and Molecular Biology*, **8**, 115 (1968).
- 13) C. A. Hutchison, III, and R. L. Sinsheimer *J. Mol. Biol.*, **18**, 429 (1966).
- 14) D. H. Duckworth, *Bacteriol. Rev.*, **34**, 344 (1970).