Selective Inhibition by Pantoyl Lactone and Butyl Alcohol of the Initiation of DNA Replication in *E. coli*

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We studied action mechanisms of pantoyl lactone and butyl alcohol on the macromolecular synthesis of *E. coli*. Protein synthesis was not significantly suppressed by these agents. DNA synthesis was more remarkably affected than RNA synthesis by them. Synchronous cultures of *E. coli* were subsequently used to investigate the inhibition of DNA replication with these agents. It was consequently shown that these agents inhibit the initiation of a new cycle of DNA replication in this organism but permit the completion of DNA replication initiated before addition of these agents to the medium.

Very low doses of UV-light cause *E. coli* B as well as *lon*⁻ mutants of *E. coli* K-12 to grow into long, multinucleate, nonseptate and filamentous cells on rich media, therefore preventing formation of macrocolonies.^{1,2)} It is well known that this kind of UV-damage is reversed by pantoyl lactone,³⁾ but the mechanism involved is not known. Our previous studies have shown that phenethyl alcohol and butyl alcohol possess division-stimulating activities which are similar to those found with pantoyl lactone.⁴⁾

Treick and Konetzka⁵⁾ reported that phenethyl alcohol inhibits the initiation of a new cycle of DNA replication in *E. coli* but permits the completion of DNA replication initiated before addition of this agent into the medium. Learning from these results, we examined the effect of pantoyl lactone and butyl alcohol on the macromolecular synthesis of *E. coli*. It was found that these agents inhibited most markedly DNA synthesis among macromolecular synthesis examined and blocked selectively the initiation of DNA replication.

MATERIALS AND METHODS

Bacterial growth. *E. coli* W2252 (thy⁻, met⁻) (given by Dr. T. Beppu, University of Tokyo) was incubated at 37°C with aeration in the following medium. Medium GT; glucose 2 g, KCl 2.02 g, NH₄Cl 1.98 g, CaCl₂ 14.7 mg, MgCl₂ 142 mg, Na₂SO₄ 398 mg, FeCl₃ 2.7 mg,

Tris-HCl 12.1 g, thymine 30 mg, methionine 50 mg per liter; the pH was adjusted to 7.6.

Protein and nucleic acid syntheses. Cells of *E. coli* W2252 grown overnight in the medium GT were transferred into 50-fold volumes of fresh medium and incubated at 37°C. At the logarithmic phase of growth, the cell suspension was divided into 9.7 ml portions in L-tubes at 0°C. After 10 min of incubation at 37°C, 0.5 μC of ¹⁴C-amino acids or 0.5 μC of ¹⁴C-adenine was added simultaneously with 50 μg of adenine to each culture. At the same time, pantoyl lactone or butyl alcohol was supplemented into the culture. Nucleic acids and protein were fractionated from 3 ml of samples according to the method of Schmidt and Thannhauser.⁶⁾ The radioactivities of the fractionated materials were determined with a GM counter.

Synchronous cultures. Synchronous bacterial cultures were prepared by the method described by Cutler and Evans.⁷⁾ The cells of *E. coli* W2252 grown overnight in the medium GM were transferred into fresh medium and incubated at 37°C. Cells were harvested by membrane filter (Sartorius, 0.45 μ) at 15 min after reaching the initial stationary phase as controlled by optical density measurements, and were then incubated into fresh medium. The above procedure was repeated once more. Subsequent cultures exhibited stepwise cellular divisions as expected for synchronous cultures. The number of viable cells was determined by colony-formation on nutrient agar plates consisting of 7 g of meat extract, 10 g of peptone, 3 g of NaCl per liter, pH 7.0.

¹⁴C-thymine incorporation. Synchronized cells were transferred into the medium GT supplemented

with $0.03 \mu\text{C}$ per ml of ^{14}C -thymine and were incubated at 37°C with shaking. Aliquots (1 ml) were withdrawn at intervals and combined with 1 ml of 10% cold trichloroacetic acid. After standing for 60 min in the cold, cells were centrifuged at $3000 \times g$ for 10 min and washed with an equal volume of cold trichloroacetic acid. After the precipitates were collected and suspended in 1 ml of 1 N KOH, the radioactivity was counted by a Horiba Liquid Scintillation spectrometer.

RESULTS

Effects of pantoyl lactone and butyl alcohol on the macromolecular synthesis of E. coli.

The effect of pantoyl lactone on protein and nucleic acid synthesis in *E. coli* W2252 cells grown exponentially in culture is illustrated in

FIG. 1. Effect of Pantoyl Lactone on the Macromolecular Synthesis of *E. coli*.

After addition of radioactive materials, the culture was divided into three parts; control (●—●), 0.10 M pantoyl lactone added (×—×), 0.15 M pantoyl lactone added (○—○).

FIG. 2. The Effect of Butyl Alcohol on the Macromolecular Synthesis of *E. coli*.

closed circles, control; open circles, 0.8% butyl alcohol

Fig. 1. RNA synthesis was somewhat more sensitive to pantoyl lactone than protein synthesis. It is also shown that DNA synthesis was more remarkably affected than RNA synthesis. Pantoyl lactone inhibited, at a concentration of 0.10 M protein, RNA and DNA synthesis by zero, 16 and 48%, respectively. We then examined the effect of butyl alcohol on the macromolecular synthesis of *E. coli*; this agent recovers the UV-damaged cell division of *E. coli* B like pantoyl lactone. It was observed that DNA synthesis was most sensitive to butyl alcohol as in the case of pantoyl lactone (Fig. 2).

Inhibition of DNA synthesis with pantoyl lactone or butyl alcohol

We then studied the effect of pantoyl lactone or butyl alcohol on DNA replication in synchronized cells of *E. coli* W2252 obtained

FIG. 3. The Effect of Pantoyl Lactone on DNA Synthesis of *E. coli* Growing Synchronously.

(A) 0.1 M pantoyl lactone was added to the synchronous culture of *E. coli* at 0 (○), 20 (△) and 40 (□) min. A culture without the drug is indicated by closed circles.

(B) Growth curve of a synchronous culture of *E. coli*.

FIG. 4. The Effect of Butyl Alcohol on DNA Synthesis of *E. coli* Growing Synchronously.

Synchronized cells were transferred into the medium supplemented with ^{14}C -thymine and were incubated at 37°C . 1% butyl alcohol was added to the culture at 0($\circ$), 20(Δ) and 40($\square$) min. A culture without the drug is indicated by closed circles. Aliquots were withdrawn at intervals and the radioactivities were measured.

by the stationary-phase synchrony method.⁷⁾ As indicated in Fig. 3-B, the cells were synchronized with a generation time of 45 min. As can be seen in Fig. 3-A, if pantoyl lactone was added into the synchronous culture at zero time and 20 min, DNA synthesis was prevented at 25 min, while the addition of the agent at 40 min allowed DNA synthesis up to 65 min. Cells of *E. coli* growing in a glucose medium with a generation time of 45 min possess chromosomes that are almost half-replicated.⁸⁾ Considering these findings, the continuation of DNA synthesis in the presence of pantoyl lactone suggests that the compound might not affect the polymerization involved in DNA replication, once the process had been initiated. As shown in Fig. 4, it appears that butyl alcohol also inhibits the initiation of a new cycle of DNA replication like pantoyl lactone. It may be very probable that a strict range of drug concentration might be required to inhibit the initiation of DNA replication. Concentrations lower than the

critical one may allow the initiation of DNA synthesis; concentrations greater than the critical one may markedly inhibit the synthesis of macromolecules and therefore kill the cell.⁴⁾

DISCUSSION

It has been known that the UV-damage is reversed by pantoyl lactone. The present results indicated that pantoyl lactone and butyl alcohol affected most remarkably the DNA synthesis among macromolecular syntheses studied in *E. coli* W2252. Furthermore, it was demonstrated in synchronized cultures that these agents inhibit initiation of a new cycle of DNA replication in *E. coli* but permit the completion of DNA replication initiated before addition of these agents into the medium.

The UV-exposed cells recover their division ability by the addition of phenethyl alcohol⁴⁾ which is known as an inhibitor of DNA synthesis. In *E. coli*, phenethyl alcohol has been reported to permit the completion of DNA replication but to inhibit the initiation of new DNA replication cycle.⁵⁾ Contrary to such an effect of phenethyl alcohol, several other inhibitors of DNA synthesis such as mitomycin C, bleomycin and nalidixic acid have been known to inhibit DNA synthesis of bacteria immediately after addition of chemicals into the culture at any time of the cell cycle^{9~11)} and to cause the filament formation of bacteria.^{10,12,13)} These agents, at concentrations tested, did not stimulate cell division of UV-irradiated *E. coli* B but suppressed the formation of colonies.

Considering the above informations as well as the present results, it may be concluded that the chemicals such as pantoyl lactone, butyl alcohol and phenethyl alcohol stimulate or recover the cell division ability in UV-irradiated cells of *E. coli* B by suppressing selectively the initiation of DNA replication.

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