SHORT COMMUNICATION

PHOTODYNAMIC INACTIVATION OF ESCHERICHIA COLI CELLS AFTER STARVATION FOR REQUIRED AMINO ACID OR CHLORAMPHENICOL TREATMENT

SHINJI MATSUMOTO

Division of Chemistry, National Institute of Radiological Sciences, Chiba 280

Received November 1, 1973

It has been reported that the physiological state of bacterial cells at the time of X- or UV-irradiation (Billen 1963; Hanawalt 1966; Billen and Bruns 1970) modifies their lethal response. These results have been discussed in relation to repair synthesis and physiological state of the chromosome which was synthesized during prevention of protein synthesis. In the present communication, physiological modifications of the photodynamic inactivation rate with acridine orange by prevention of protein synthesis are described. It is noted that prevention of protein synthesis by required amino acid deprivation makes the cell resistant to photodynamic inactivation in contrast to chloramphenicol treatment.

A strain of E. coli, 15 TAU (thy−, arg−, ura−), was used. This was originally isolated in the laboratory of Dr. S. S. Cohen. Bacteria were grown in a minimal glucose-salt medium (McCarthy 1962) supplemented with nutritional requirements. Minimal medium without glucose was used as buffer. Acridine orange was added to the suspension of washed cells in buffer and then the mixture was illuminated. Surviving fractions after illumination have been compared for cells in exponential growth and those after incubation in buffer, in the amino acid deprived medium or in the complete medium supplemented with chloramphenicol. The incubation time of 90 min was based on the references (Billen 1963; Hanawalt 1966; Billen and Bruns 1970).

The results shown in Fig. 1 indicate that the cells which had been incubated in the amino acid deprived medium gained an enhanced resistance as compared to those in exponential growth. On the contrary, the cells pre-treated with chloramphenicol became more sensitive than those in exponential growth; this is a similar degree of sensitivity to the cells suspended in buffer for the same period. When chloramphenicol was added to amino acid deprived medium, the surviving fraction decreased to the level of chloramphenicol alone, and showed a similar degree of the survival to that of the chloramphenicol treatment alone. Therefore, the chloramphenicol addition overwhelmed the physiological modification caused by amino acid starvation and prevented the cells to acquire the resistant state. These experiments were repeated several times and the reproducibility was confirmed. In the case of stationary phase cells, they were not
Fig. 1. Effect of different pre-incubations on photodynamic inactivation of E. coli 15 TAU strain. Cultures were grown in minimal glucose-salt medium, supplemented with 2 μg thymidine, 40 μg L-arginine, and 20 μg uridine per ml. Exponentially growing cultures were washed by centrifugations and divided into four portions. One portion (×) was suspended in buffer and acridine orange was added at a concentration of 5 μg/ml to a bacterial suspension of 3x10⁸ cells/ml. The mixture was shaken moderately for 20 min at 37°C. Other three portions were treated in the same procedure, but after different pre-treatments such as incubation in the buffer (△), incubation in complete medium without required arginine and uridine (○), or incubation in complete medium with chloramphenicol (●). All the above incubation were done for 90 min at 37°C. The cells were then illuminated with a photoreflector lamp (500 watt) at a distance of 11.5 cm in an ice bath. The light intensity was monitored by a photocell illuminometer (Toshiba, SPI-5). Following illumination, the cells were plated on nutrient agar plates and visible colonies were scored after incubation for 2 days at 37°C.

inactivated in the same range of illumination time even after amino acid starvation or chloramphenicol treatment, according to the data that are not shown here.

Since different sensitivities caused by amino acid starvation and chloramphenicol treatment seemed to be particularly interesting, similar experiments were carried out
PHOTOINACTIVATION OF ACRYDINE-SENSITIZED E. COLI CELLS

with strains of known radiation sensitivities such as AB 1157 (thr−, leu−, prol−, his−, arg−, Bl−, uvr+A), AB 1886 (thr−, leu−, prol−, his−, arg−, Bl−, uvr−A), JC 1557d (leu−, his−, arg−, met−, rec+A), and JC 1569b (leu−, his−, arg−, met−, rec−A). The results are shown in Fig. 2. Again, only the cells pre-incubated in the amino acid deprived medium became markedly resistant, but the effect was rather moderate in the rec−A strain. The survival rate of JC 1557d (rec+A) was similar to that of AB 1157 and not shown here. The survival rate of chloramphenicol treated cells (Fig. 2) was of a similar degree to that of the cells in exponential growth, however, the unique susceptibilities under the two conditions were difficult to determine, since the difference was very small.

Acridine orange-sensitized photodynamic inactivations of X- or UV-sensitive K12 strains (Rupp 1966; Harm 1968; Janovská et al. 1970; Harrison Jr. et al. 1972) have been discussed in terms of their repair function. In the present case, the cellular resistance gained by amino acid starvation was not modified by the uvrA gene but influenced by the recA gene. Therefore the high survival rate after amino acid starvation may be partially related to the recA function, though the mechanism involved is not clear. Since the recA gene is responsible for rec-dependent repair of damaged DNA, the degradation of DNA has been investigated to examine the possibility of DNA as the target material of the lethality. TCA-insoluble counts of the fully labeled DNA after illumination has been obtained for thymine requiring strains of JC 1557d and JC 1569b. Results are shown in Fig. 3. Since the rates of DNA degradation were large in the rec−A strain and in the chloramphenicol treated rec+A strain, the DNA degradation

Fig. 2. Effect of different pre-incubations on photodynamic inactivation of AB 1157 (uvr+A), AB 1886 (uvr−A), and JC 1569b (rec−A) of E. coli K12. Experimental conditions were similar to that of Fig. 1, except that cell concentration was 1×10^6 cells/ml. ×, untreated, exponentially growing cells; ○, cells incubated in the leucine deprived medium prior to the treatment; or ●, cells incubated in complete medium with chloramphenicol prior to the treatment.
associated with acridine orange photosensitization might be related to the main lethal process.

The present results indicate that the cells pre-incubated in amino acid deprived medium possess enhanced resistance to photodynamic inactivation in contrast with those pre-incubated with chloramphenicol. This effect of the amino acid starvation seems to be similar to that described by others with X- or UV-inactivation kinetics (Billen 1963; Hanawalt 1966; Billen and Bruns 1970). However, in our case, the addition of chloramphenicol did not result the resistant state like the amino acid starvation. The different susceptibility after amino acid starvation or chloramphenicol treatment may be partially related to the recA function but it may arise from other causes too. In the case of stationary phase cells of 15 TAU strain, they were resistant to photodynamic inactivation in comparison with those in exponential phase. Since it has been well known that the rate of uptake of various compounds from the surrounding medium into a cell is usually lower value in stationary phase, we suspect that cellular uptake of acridine orange might be also low in the stationary phase cells of 15 TAU. Although there

Fig. 3. $^3$H-radioactivity remaining in acid-precipitable form expressed as a percentage of the initial level, during incubation after illumination. Results are given for the thymine requiring derivatives of JC 1557d (rec$^+$A) and JC 1569b (rec$^-$A). Cells were labeled in their DNA by growth with $^3$H-Tdr for several generations. Experimental procedures were similar to those adopted in the survival measurement, except that acridine orange was added at a concentration of 10 $\mu$g/ml to bacterial suspensions of 5 x 10$^7$ cells/ml and the time of illumination was 25 min. ●, rec$^+$A, pre-incubated in the leucine deprived medium; ○, rec$^+$A, pre-incubated in the complete medium with chloramphenicol; ▲, rec$^-$A pre-incubated in the leucine deprived medium; △, rec$^-$A, pre-incubated in the complete medium with chloramphenicol.
should exist several types of modification such as interaction of acridine orange with ribosomes, the most important possibility might be a change in the membrane permeability, as pointed out by Sugino (1966) in a mutant sensitive for acridine orange. The cause of the resistant state gained by amino acid starvation might be also explained by supposing a change in the membrane permeability caused by alteration in the protein turnover in amino acid-starved cells. As a matter of fact, it has been known that labile part of the cell membranes become preferred sites of turnover on sufficient starvation (Pine 1966). Since the effect of amino acid starvation on the enhancement of cellular resistance is so large, this may well explain large variations of inactivation rates in radiation sensitive cells in exponential phase as observed by several investigators (Rupp 1966; Harm 1968; Janovská et al. 1970; Harrison Jr. et al. 1972) even in consideration with probable different rates of protein synthesis according to their experimental conditions.

SUMMARY

Acridine orange-sensitized photodynamic inactivation of E. coli cells has been investigated under different physiological conditions. It was found that the cells pre-incubated in amino acid deprived medium became most markedly resistant in contrast to those pre-treated with chloramphenicol. The cellular resistance gained by amino acid starvation was not modified by the uvrA gene but the effect was rather moderate in the rec^A strain.

ACKNOWLEDGMENT

The author thanks Dr. T. Ito for valuable comments on the manuscript and Mrs. Y. Kagami-ishi for her technical assistance.

LITERATURE CITED