λ - PHAGE INDUCTION BY COLICIN E_2*

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With respect to the mechanism of the prophage induction, many authors have presented evidences indicating that the specific inhibition of DNA synthesis in lysogenic bacteria might hold the key to the phenomenon (Sekiguchi and Takagi, 1960; Melechen and Skaar, 1962; Endo, Ishizawa, Kamiya and kuwano, 1963). From this point of view an attempt has been made by us to approach the nature of the mechanism through some influences which affect the DNA metabolism in bacterial cells. Recently Nomura et al. (1962) reported the reversible inhibitory action of phage T_4 and colicin K on nucleic acids and protein synthesis in E. coli. This led us to test the prophage inducing activity of colicins on the lysogenic strain E. coli K-12. We give here some results dealing with the prophage-inducing action of colicin E_2 and preliminary biological features of this system.

Materials and Methods For these studies we have used E. coli K-12 (λ) and a streptomycin resistant indicator strain E. coli C-600 which were kindly supplied by Dr. J. Tomizawa of the National Institute of Health, Japan. Colicin E_2 was prepared in accordance with the unpublished procedure by Dr. Ozeki. An overnight culture of S. typhimurium cys-36 (E_2) was irradiated with UV at the dosis of 80 % killing, and incubated with vigorous aeration in

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a pepton broth. After 3 to 4 hours culture, when the production of the colicin reaches maximal value, a small amount of $CHCl_3$ was added, and the lyzate was refrigerated overnight. The cellular debris was removed by centrifugation and solid $(NH_4)_2SO_4$ was added to the supernatant to 60 % saturation. The precipitate was collected by centrifugation, dissolved in a small amount of water, and dialyzed overnight against 0.005 M phosphate buffer (pH 7.0). The dialyzate was applied to a column of DEAE-cellulose equilibrated against the same buffer as that used in the dialysis. Unadsorbed fraction was collected, dialyzed and lyophilized. The final preparation showed a specific activity (Goebel and Barry, 1958) about 6,000 times that of the original lyzate. Trypsin employed in the present experiment is a crystallized sample of Worthington Biochemical Corp.

E. coli K-12 (λ) was grown and harvested at a logarithmic phase by centrifugation to remove free phages and resuspended in a fresh λ-broth consisting of 1 % polypepton and 0.5 % NaCl. The cells were contacted with colicin E_2 for 5 minutes at 37°, then quickly diluted 10^{-4} to 10^{-5} fold and plated with or without indicator strain E. coli C-600 Sm^r for the estimation of induced cells and survivals respectively. For the estimation of induced cells, 0.1 ml of streptomycin (10mg/ml) was spread on the bottom of agar 2 hours after plating. This procedure is able to eliminate satisfactorily the spontan-induction of survived E. coli K-12 (λ).

Results and Discussion The conditions for the phage induction of E. coli K-12 (λ) by colicin E_2 are shown in Table I. From these experiments it became clear that in the following three conditions λ - phage formation can be induced.

1. Colicin E_2 can induce E. coli K-12 (λ) in a relatively low concentration without any other treatments. In this condition, the adsorption of the colicin to the bacteria leads to one of two events; the induction of the prophage λ or the death of the cell. Their fate seems to depend on the number of the colicin molecule adsorbed on the cell (unpublished data).

2. Inducing action of this agent is also shown at relatively high concentration but only when followed by treatment with trypsin. In this con-

Table I

Conditions for the phage induction in *E. coli* K-12 (λ) by colicin E_2

Experiment	Treatment	No. of Induced	No. of Survival
1	K-12 only	8×10^5	1.3×10^8
2	E_2 only	no plaque	no colony
3	Trypsin only	"	"
4	Trypsin + E_2	"	"
5	K-12 + Trypsin	6×10^5	1.5×10^8
6	K-12 + diluted E_2	2.3×10^7	5.3×10^7
7	K-12 + E_2	7.7×10^6	3.0×10^6
8	K-12 + E_2, then trypsin	3.0×10^7	5.3×10^7
9	Trypsinated K-12 + E_2	3.0×10^7	1.7×10^7
10	Trypsinated E_2 + K-12	4×10^5	2.9×10^8

Growing K-12 (λ) washed and resuspended to 2×10^8 cells /ml in broth was added to an equal volume of the broth containing trypsin (experiment 5) or colicin E_2 (experiment 6, 7 and 8). In experiment 8 colicin-treated cells were diluted 10^{-2} fold and treated further with trypsin. In experiment 9 trypsinated K-12 was prepared by incubating the cells in the broth containing trypsin, then washed two times by centrifugation, and treated further with colicin E_2 as experiment 6. In experiment 10, colicin E_2 was treated with trypsin and diluted so as to give a corresponding concentration to that used in other experiments. Trypsination was carried out in the concentration of 1 mg/ml for 20 minutes, and colicin E_2 was interacted with the cells for 5 minutes throughout the experiment. The action of these agents was terminated by dilution. All the samples were plated after 25 minutes from the beginning of the procedure. Colicin titer used in the present experiments was 97 % killing/5 minutes except that of No. 6, in which 50 % killing/5 minutes dosis was used. All procedure was carried out at 37°.

dition, almost all the cells in the population adsorb colicin promptly and are killed as the result. When trypsin was added to such an inhibited culture, a considerable number of the cell was transformed into induced cells and the rest revived. The optimal concentration of trypsin was 1 mg/ml at 37° for 20 minutes. Trypsin can be replaced with pronase (Kaken-Kagaku Co.).

3. When the cells were pretreated with 1 mg/ml of trypsin for 20 minutes before the treatment with colicin E_2 and were thoroughly washed, high concentration of colicin E_2 can induce phage formation in *E. coli* K-12 (λ). Thereby, apparent resistance of these cells for the colicin seems to increase.

We checked that the observed increase in plaque number in the three conditions described above is really due to the induction of prophage from the

lysogenic host, by isolating the phage from the plaques and examining host-range relation. The obtained phages were capable of infecting E. coli C-600 but not E. coli K-12 (λ) or E. coli C-600/λ. The plaque morphology was the same as that of parent plaques. These results exclude the possibility of contamination in our colicin preparations not only of any other phages but also of colicinogenic bacteria.

By treating the cells with colicin E_2 of high concentration followed by the treatment with trypsin, a typical one step growth curve of λ-phage was obtained (Fig. I). It may be seen that the latent period is 50 minutes and the average burst size is 89. The pattern of the induction is essentially the same as that by UV or mitomycin C.

Colicin E_2 used in this experiment was a partially purified one, and a possibility remains that the inducing action and colicin activity which is manifested by the killing action for E. coli K-12 (λ) originate in the different molecular entities in the preparation. The following experiments seem to rule out this possibility and support the idea that the inducing action is the expression of colicin E_2 itself: (a) The ratio of both activities remains constant during the purification procedure. (b) E. coli K-12 (λ)/E_2 could not be induced by the action of our preparation. (c) E. coli K-12 (λ)(E_2) which is immune to the killing action of colicin E_2 but capable of adsorbing this colicin is also insensitive to the action of our preparation.

The results of this investigation indicates that colicin E_2 can induce λ-phage from lysogenic E. coli K-12. The fact that colicin K and phage T_4, after the adsorption to the sensitive host, prevent nucleic acids and protein synthesis presumably inhibiting DNA function of the host cells (Nomura et al. 1962), suggests that the inhibition of DNA metabolism by colicin E_2 might also play a fundamental role in the induction of the prophage.

As for the role of trypsin in the present experiment, it is readily conceivable that this proteolytic enzyme serves to favor the phage inducing effect of colicin E_2 by digesting an excess amount of the colicin. It seems thus very likely that trypsin itself does not play a direct role for the prophage induc-

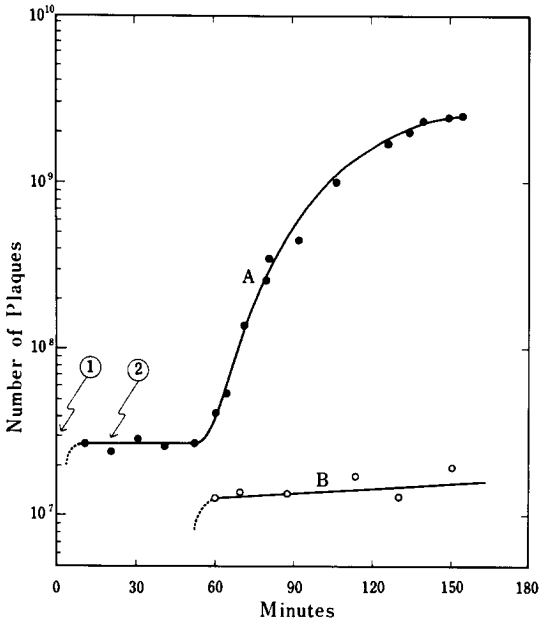

Figure 1. One step growth in phage induction in E. coli K-12 (λ) by colicin E_2

The washed cells were contacted with colicin E_2 in the concentration of 8.7×10^7 cells/ml for 5 minutes. At the end of the treatment, trypsin was added to the cell suspension so as to give a final concentration of 1 mg/ml (arrow 1) and incubation was continued further for 20 minutes. At the end of the incubation, the suspention was diluted to the appropriate concentrations (arrow 2). Up to 160 minutes, samples were taken and plated. Colicin titer used was 94 % killing/5 minutes dosis. All procedure was carried out in broth at 37°. Curve A, treated; Curve B, nontreated.

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tion. These considerations are strongly supported by the fact that in a certain condition, the prophage is inducible by the treatment with colicin E_2 only.

The inducing action of colicin E_2 led us immediately to test whether the other colicins also show the same inducing action. It was found that colicin K and E_3, inspite of repeated experiments, failed to reveal any inducing action. Colicin E_2 and E_3 are known to be adsorbed on the same site of the bacterium. These results might be the manifestation of the difference in the property of the colicin to affect the lysogenic bacterium. The different explanation is that the active center of colicin E_2 for the prophage inducing action and for the killing action may reside in the different site on the same molecule. Further works are in progress to elucidate these points.

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