EFFECT OF DESOXYRIBONUCLEASE ON COLICIN D PRODUCTION BY Escherichia coli STRAIN CA-23 (D)

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The increase in colicin D production by Escherichia coli strain CA-23 (D) in the presence of desoxyribonuclease in a solution containing magnesium is due to the effect of magnesium ions and not of the enzyme. Incubation of cells irradiated with x-rays in a solution with an increased magnesium concentration leads to a decrease in the number of cells producting colicin.

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It is now known that the intracellular development of temporate phage is accompanied by an increase in nuclease activity [6, 7, 13, 15]. The fact that incubation of intact lysogenic cells with solutions of ribonuclease (RNase) [11] or desoxyribonuclease (DNase) [1, 2] facilitates the development of temporate phage has led some workers to postulate that activation of intracellular nucleases by an inducing agent acts as the trigger for the mechanism of induced development of temporate phage [1, 2, 11, 13]. So far as the colicinogenic bacteria are concerned, no information is yet available.

On the assumption that the same agent can effectively act as inducer in lysogenic and colicinogenic systems, the present investigation was carried out to study the effect of solutions of RNase and DNase on the ability of colicinogenic strains to produce colicin.

EXPERIMENTAL METHOD

The investigation was carried out with strain Escherichia coli Ca-23 (D), producing colicin D. A sample of the culture was incubated with the enzyme (pH of solution 6.8-7.2) with slow stirring at 37°. The number of viable cells producing colicin in the samples was determined after 10, 30, 60, and 120 min. The technical details mentioned in [10] were followed when determining the results.

EXPERIMENTAL RESULTS

During cultivation of intact cells of E. coli strain CA-23 (D) with RNase solution (10 and 100 μg/ml) for various periods of time, no changes could be found in the level of spontaneous colicin production. No change in the level of colicin production took place either with a change in the enzyme concentration, incubation temperature, pH of the medium, or an increase in the incubation time.

Results showing the effect of DNase solution in 0.01M magnesium solution are given in Table 1. As this table shows, the presence of 10 μg/ml of DNase in the medium, dissolved in 0.01M magnesium solution, stimulated colicin synthesis. The effect reached a maximum after incubation for 30 min. However, the presence of magnesium alone in the medium produced a more marked induction effect, which also reached a maximum after incubation for 30 min. This fact, and also that when other parameters were used to determine the degree of induction produced by DNase solution in 0.01M magnesium solution, it was always lower than that due to magnesium solution alone, suggest that the inducing action of the enzyme solution was apparently associated with the presence of magnesium in the medium. This is also confirmed by the fact that an increase in concentration of the enzyme to 100 μg/ml in the same volume of 0.01M magnesium solution did not increase but, on the contrary, decreased the degree of induction. This was evidently due to adsorption of magnesium by the enzyme from solution.

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TABLE 1. Effect of DNase on Colicin Production by E. coli Strain CA-23 (D)

Incubation time (in min)	Control culture	Culture + 10 μg/ml DNase in 0.01M magnesium solution	R	Culture + 0.01M magnesium solution	R
		Number of cells producing colicin (× 10⁴)			
10	0.407±0.018	0 421±0.021	1.03	0,592±0.034	1.45
30	0.319±0.022	0.464±0 019	1.45	0.645±0.051	2.02
60	0.45±0.01	0.519±0.029	1.15	0.669±0.028	1.48
120	0.381±0.027	0.457±0.03	1.19	0.545±0.024	1.43
		Number of viable cells (in %)			
10	100	84.6±1.4		103.9±0.9	
30	100	103.2±0.6		76.6±1.3	
60	100	95.5±1.1		76.7±1.8	
120	100	75.05±2.3		97.4±0.7	

Note. R denotes number of times greater than control (degree of induction).

TABLE 2. Effect of DNase Solution in 0.01M Magnesium Solution on Ability of X-Ray Irradiation to Induce Colicin Synthesis in E. coli Strain CA-23 (D)

Incubation time (in min)	Culture + irradiation	Culture + irradiation + 10 μg/ml DNase in 0.01M magnesium solution	R	Culture + irradiation + 0.01M magnesium solution	R
		Number of cells producing colicin (× 10⁴)			
10	5.62±0.32	3.94±0.3	0.7	3.07±0.27	0.55
30	6.78±0.51	3.89±0.35	0.45	3.2±0.29	0.37
60	5.17±0.23	4.35±0.31	0.84	3.7±0.24	0.72
		Number of viable cells (in %)			
10	7.5±0.4	8.59±0.21		11.6±0.46	
30	6.61±0.37	7.2±0.26		10.3±0.78	
60	4.5±0.2	5.5±0.3		7.5±0.64	

Note. R denotes number of times greater than control (degree of induction)

The absence of effect of the enzyme on colicin production in colicinogenic cells can be explained as follows: 1) the enzyme did not penetrate into the colicinogenic cells, 2) the enzyme did penetrate but was inactivated, 3) the enzyme penetrated and was not inactivated, but colicinogenic cells do not contain the substrate on which the enzyme acts and which is responsible for induction (for example, the prophage–bacterial chromosome link).

The possibility of penetration of the enzyme molecule into the bacterial cell would seem to be slight. First, in that case it would have to be accepted that the bacterial cell has no defence reactions. Second, investigations have shown that the enzyme macromolecule cannot penetrate, not only into intact cells [4-6, 10], but also into protoplasts [5, 9]. Third, the presence of an increased magnesium concentration in the cultivation medium must lead to stabilization, to condensation of the cell memebranes [14], and this must also to some extent prevent penetration of the enzyme into the cell.

The enzyme penetrated into the cell but was inactivated. In this case it would have to be accepted that this could only be due to the presence of colicinogenic factor in the cell. Increased resistance of colicinogenic cells might then be due to the fact that either the action of the enzyme was inhibited by colicin molecules or the enzyme reacted with colicinogenic factor and while destroying it became inactivated.

However, the fact that an increase of ten times in the enzyme concentration reduced the inductive effect is evidence against the first possibility. On the other hand, in none of our experiments was it possible

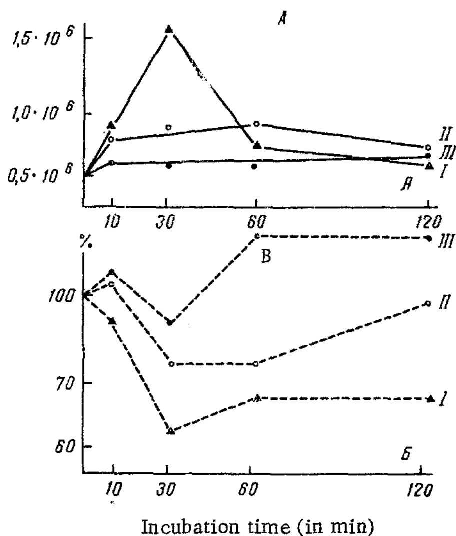

Fig. 1. Effect of different concentrations of $MgSO_4$ on production of colicin D by strain E. coli CA-23 (D). A) number of "lacunae"; B) percentage of viable cells; I) culture + 0.1M $MgSO_4$ solution; II) culture + 0.01M $MgSO_4$ solution; III) culture + 0.001M $MgSO_4$ solution.

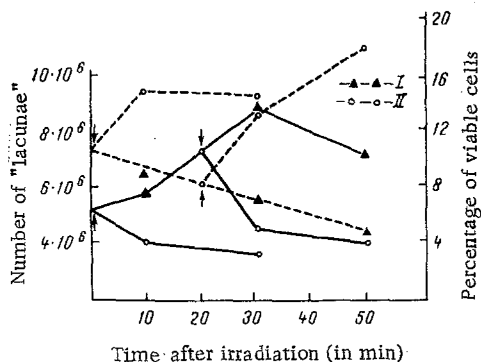

Fig. 2. Effect of 0.1M $MgSO_4$ solution on synthesis of colicin D induced by x-ray irradiation. I) culture + irradiation; II) culture + irradiation + 0.1M $MgSO_4$ solution. Time of addition of magnesium marked by arrow.

to observe elimination of colinogenic properties under the influence of DNase. The absence of high specificity in the action of the enzyme also rules out the last possibility: action of the enzyme on a certain cell substrate responsible for the onset of induction.

For final confirmation that the inductive action of DNase solution in 0.01M magnesium solution was due to the presence of magnesium, a series of experiments was carried out on the basis of the following hypothesis. If, by analogy with lysogenic cells, in colicinogenic cells exposed to the action of the inducing agent an increase in nuclease activity must in fact be observed, and if these nucleases are triggers in the chain of induced colicin synthesis, as a result of the combined action of x-ray irradiation and DNase, the induction effect must be increased.

For this purpose a sample of the culture was irradiated with x-rays (10 000 R) and enzyme was added immediately after irradiation. The number of viable cells and the number of cells producting colicin were counted in the cultures after 10, 30, and 60 min. The results obtained are given in Table 2.

It is clear that the presence of the enzyme DNase in the culture medium of the irradiated cells did not increase but, on the contrary, decreased the induction effect. However, as in the preceding experiments, the main action of the solution was due to the presence of magnesium, which stimulated the repair of damge induced by irradiation.

The fact that the inductive action of DNase solution in 0.01M magnesium solution is due to the magnesium formed the basis for a study of the effect of magnesium on spontaneous (Fig. 1) colicin production and colicin production induced by x-ray irradiation (Fig. 2).

As the results illustrated in Fig. 1 show, the ability of magnesium to induce colicin synthesis depends on the magnesium concentration and the incubation time. The higher the concentration, the shorter the time required to reach the maximum of induction. The number of viable cells also changed with the magnesium concentration in solution. The greatest decrease in the number of viable cells took place at 35 min of incubation, and the higher the magnesium concentration, the greater this decrease and the longer it lasted. This period was followed by stimulation of cell devision.

The results given in Table 2 illustrate the effect of magnesium on colicin production induced by x-ray irradiation. It is clear that the action of magnesium on irradiated cells consisted essentially of stimulation of repair of damage induced by irradiation. This effect was most marked when magnesium was added 20 min after irradiation and it usually was demonstrable during the first 10 min of cultivation.

The results of these experiments thus suggest that treatment of intact cells of colicinogenic strain CA-23 (D) with RNase or DNase solution does not affect the level of spontaneous colicin production, and treatment with DNase likewise does not affect the level of induced colicin production. The ability of DNase in 0.01M magnesium solution to stimulate colicin production is connected with the presence of magnesium ions in the solution. The inductive action of magnesium is directly dependent on the concentration and the incubation time. The ability of magnesium to induce colicin synthesis is evidently unconnected with activation of intracellular nucleases, as follows from the results of experiments in which cells were exposed to

the combined action of x-ray irradiation and magnesium. The addition of magnesium to irradiated cells stimulates the repair of injuries induced by x-ray irradiation. This repair effect is most marked when magnesium is added at the beginning of the second half of the latent period of colicin synthesis.

The increase in nuclease activity observed during the development of temporate phage in a lysogenic cell must evidently be regarded as an example of reorganization of cell metabolism under the control of the virus genome [3, 8], and not as a trigger mechanism in the chain of induced synthesis.

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