THE EFFECT OF CYSTAMINE ON INDUCED PHAGE PRODUCTION IN RELATION TO CULTURE CONDITIONS OF LYSOGENIC BACTERIA

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In connection with the search for models for the study of radiation shielding properties of various chemical compounds the use of lysogenic bacteria is of great interest.

The purpose of the present work was the study of the effect of cystamine (disulfidemercamine) on induced phage production by a lysogenic strain of E. coli K-12 (γ) and to determine the relation of its level to the time the bacteria are kept in the medium with the preparation, to the concentration of the preparation, temperature and pH of the medium.

EXPERIMENTAL METHODS

Samples of four hours broth cultures of E. coli K-12 (γ) which were mixed in equal volumes with meat-peptone broth (MPB) containing cystamine were used in the investigation. The final concentration of the preparation was 0.01, 0.025, 0.05 and 0.1%. The test tubes with the bacteria and cystamine were placed in a water bath at 37° or on ice at 0°.

After various periods at these temperatures (5, 30, 60, 120 and 240 min) 0.5 ml of the culture fluid was removed from the test tubes for analysis and an equal volume of physiological solution cooled to 0° and containing cystamine in the same concentration as in the MPB added to it. The pH of the medium was determined before irradiation (5.1, 6.2, 7.2, 8.0 and 9.0). The samples were exposed to x-rays, using the RUM-7 x-ray apparatus with a dose rate of 4050R/min, voltage 50 kV current strength 155 mA and distance from the source 8 cm (0.1 mm thick aluminum filter). Analogous sample, but without cystamine, were used as controls. After irradiation the number of induced bacteria, both in the experimental and in the control samples was determined by the straight line method (2). A strain of E. coli C-85 was used as the test organism. Parallel inoculations were carried out to determine the number of viable cells in the irradiated samples.

In addition, a series of experiments were carried out to determine the effect of cystamine on the lysogenic stability of E. coli K-12 (γ). The preparation was added to bacterial suspensions at 0° in concentrations of 0.05, 1% (mortality rate, 48% of the cells), 2% (mortality rate, 90.5%) and 3% (mortality rate, 96.7%) and, after 120 min, inoculations of the samples were made on MPA with subsequent examination of the colonies for lysogenicity (replica method). Colonies, about which there was doubt as to whether they came from cells which lost lysogencity, were removed, inoculated into MPB and the culture then studied for spontaneous and induced phage production. In spite of the extent of the investigations, we did not succeed in observing colonies devoid of lysogenicity.

We also studied the effect of cystamine on the length of the latent period and the yield of phage particles (γ) from one infected cell of the E. coli C-85 indicator strain (Adams method, 3) and one induced lysogenic cell of E. coli K-12 (γ) strain. In the first case the preparation was added only to the first and second growth tubes. In the second case E. coli K-12 (γ) cells induced with a 15,000R dose of x-rays and diluted to a fixed concentration were placed in a 37° water bath in test tubes with cystamine added. The final concentration of the preparation was 0.01, 0.025, 0.05 and 0.1%.

TABLE 1. Reduction of the Number of Induced Bacteria from the Effect of Cystamine in Relation to the Culture Conditions of E. coli K-12 (γ) (37°)

Incubation time (in min.)	Cystamine concentration (in %)														
	0,025					0,05					0,1				
	pH														
	5,1	6,2	7,2	8,0	9,0	5,1	6,2	7,2	8,0	9,0	5,1	,2	7,2	8,0	9,0
5	2,0	1,6	1,8	1,3	1,1	3,1	3,0	2,7	2,3	1,8	3,0	2,8	3,2	2,6	1,7
30	2,3	2,2	2,2	2,2	2,0	4,0	5,2	3,4	3,5	2,1	4,1	3,9	4,8	3,8	2,0
60	2,8	2,6	3,1	2,6	2,3	5,4	6,1	5,4	4,9	3,2	5,3	6,3	5,0	4,5	3,4
120	3,5	3,3	3,7	3,0	2,6	7,4	7,5	7,3	6,5	5,0	7,4	7,2	7,6	7,0	5,2
240	2,1	1,9	2,0	1,7	1,4	3,4	3,8	3,2	2,8	1,9	3,4	3,6	3,5	2,7	2,1

Note. Decrease in the number of induced bacteria with respect to the control is given in average arithmetical values.

TABLE 2. Dose Reduction Factors (FPD) with Respect to E. coli K-12 (γ) Culture Conditions (37°)

Incubation time (in min.)	Cystamine concentration (in %)														
	0,025					0,05					0,1				
	pH														
	5,1	6,2	7,2	8,0	9,0	5,1	6,2	7,2	8,0	9,0	5,1	6,2	7,2	8,0	9,0
5	1,7	1,5	1,6	1,2	1,0	3,0	3,0	2,6	2,0	1,6	3,0	2,7	3,0	2,5	1,6
30	2,0	1,9	1,9	1,9	1,7	4,0	7,5	3,3	3,4	1,8	4,0	3,8	6,0	3,6	1,7
60	2,7	2,5	3,0	2,5	2,0	7,5	8,0	7,5	7,0	3,0	7,5	8,0	7,0	7,0	3,3
120	3,4	3,2	3,6	3,0	2,5	10,0	10,5	9,5	8,5	7,0	10,0	9,5	10,5	9,0	7,5
240	1,8	1,7	1,7	1,6	1,2	3,3	3,6	3,0	2,7	1,7	3,3	3,5	3,4	2,6	1,8

Note. FPD values expressed in average arithmetical terms.

EXPERIMENTAL RESULTS

In Table 1 data is presented on the effect of time of contact of the bacteria with cystamine before irradiation, of the concentration of the preparation and of different pH values at 37° on induced phage production by E. coli K-12 (γ). As seen from Table 1, induced phage production depends to a large degree on the time of incubation of the lysogenic bacteria in medium with the preparation before irradiation. A maximal decrease in the number of lysogenic bacteria, induced by a 15,000R x-ray dose, is observed after 120 min in the presence of cystamine.

The effectiveness of the activity of the preparation depends on the pH of the medium, since a change in hydrogen ion concentration on either side gives different results. Thus, with a shift of pH to the acid side the effectivenes of cystamine on induced phage production does not change, but with the medium on the alkaline side the effect of the preparation is reduced. However, 240 min from the start of incubation the number of induced bacteria increases, which indicates a decrease in the anti-radiation effect of cystamine.

The induced phage production of lysogenic bacteria also depends on the cystamine concentration used. As seen from Tables 1 and 2 the largest decrease in the number of induced bacteria and the maximum dose reduction factor (FPD) is noted with the addition of 0.05% and 0.1% cystamine to lysogenic bacteria before irradiation. These concentrations do not cause a decrease in the number of viable bacteria, and the FPD value at these concentrations, calculated from the number of induced bacteria, is approximately the same. The preparation at a concentration of 0.01% does not show a noticeable effect on induced phage production in E. coli K-12 (γ).

Similar results were obtained in experiments in which the lysogenic bacteria were in contact with cystamine before irradiation at 0° (Tables 3 and 4). In this case the maximal decrease in the number of induced bacteria E. coli K-12 (γ) also occurs 120 min. From the moment the preparation is added, however this decrease was 2-3

TABLE 3. Decrease in the Number of Induced Bacteria Due to the Effect of Cystamine in Relation to Culture Conditions of E. coli K-12 (γ) (0°)

Incubation time (in min.)	Cystamine concentration (in %)														
	0,025					0,05					0,1				
	pH														
	5,1	6,2	7,2	8,0	9,0	5,1	6,2	7,2	8,0	9,0	5,1	6,2	7,2	8,0	9,0
5	1,3	1,3	1,4	1,2	1,0	1,9	1,9	1,9	1,7	1,4	2,0	1,8	1,8	1,6	1,3
30	1,9	1,8	1,8	1,5	1,2	2,4	2,5	2,7	2,2	1,6	2,4	2,2	2,2	2,0	1,6
60	2,3	2,0	2,1	1,8	1,4	2,8	2,9	3,0	2,6	1,8	2,7	2,6	2,7	2,5	1,8
120	2,8	2,4	2,5	2,2	1,8	3,9	3,5	3,5	3,1	2,8	4,1	3,4	3,6	3,2	2,6
240	1,6	1,5	1,5	1,3	1,2	2,1	2,2	2,0	1,7	1,4	2,3	1,9	2,1	1,8	1,5

TABLE 4. Dose Reduction Factor in Relation to Culture Conditions of E. coli K-12 (γ) (0°)

Incubation time (in min.)	Cystamine concentration (in %)														
	0,025					0,05					0,1				
	pH														
	5,1	6,2	7,2	8,0	9,0	5,1	6,2	7,2	8,0	9,0	5,1	6,2	7,2	8,0	9,0
5	1,2	1,2	1,2	1,1	1,0	1,7	1,7	1,7	1,6	1,2	1,7	1,6	1,6	1,5	1,2
30	1,7	1,6	1,6	1,3	1,1	2,2	2,4	2,6	1,9	1,5	2,2	1,9	1,9	1,7	1,5
60	2,0	1,7	1,8	1,6	1,2	2,7	2,8	3,0	2,5	1,6	2,6	2,5	2,6	2,4	1,6
120	2,7	2,2	2,4	1,9	1,6	3,8	3,4	3,4	3,0	2,7	4,0	3,3	3,5	3,0	2,5
240	1,5	1,3	1,3	1,2	1,1	1,8	1,9	1,7	1,6	1,2	2,0	1,7	1,8	1,6	1,3

times less than at 37°. The time of maximal decrease of phage production, as at 37°, remained constant. In exactly the same manner, when the pH was on the alkaline side, a decrease in the effect of the preparation on induced phage production was marked. The effectiveness of cystamine was clearest when the preparation was added to the bacterial suspension in concentration of 0.05 and 0.1%, while in a concentration of 0.01% its effect on induced phage production was practically absent. At concentrations of cystamine in the medium of 0.05 and 0.1% the FPD values were the same. The number of induced cells also increased in 240 min. After introduction of the preparation into the medium, that is, in this time interval the anti-radiation effect of cystamine decreased.

As was shown in the investigation, the length of the latent period does not change when the preparation is present in the medium at concentrations of 0.01 and 0.025%; however the yield of phage changes. Cystamine in a concentration of 0.01% has no effect on the amount of phage obtained, which does not go beyond the normal limits, and is 80-130 particles per induced bacterium, but in a concentration of 0.025% it reduces these figures to 3-10 phage particles per cell. With 0.05% cystamine in the medium the synthesis of phage particles (γ) in cells of the indicator strain E. coli C-85 is completely blocked (cystamine in the concentrations studied does not inactivate free phage particles).

On the basis of a series of experiments, it was established that the length of the latent period of intracellular development of induced phage (γ) in the presence of 0.01% cystamine, and also in the control, is 55 min. The yield of phage, which normally is 80-200 particles per cell, in the given case varied within the normal limits, not exceeding 160. Cystamine in a concentration of 0.025% and above completely blocked the development of phage (γ) in induced cells of the lysogenic strain.

However, after irradiation of the cells we did not observe a decrease in the number of induced bacteria kept at 0° in the medium at the concentrations of cystamine which we used.

The data obtained indicates that the anti-inducing effect of cystamine on lysogenic bacteria depends on the culture conditions of the latter (in particular, on temperature). It may be assumed that the blocking effect is linked with the penetration of the preparation into the cell which is accompanied by a definite energy discharge. Similar

results were noted by other investigators who studied in E. coli the possibility of shielding against the lethal effect of radiation by using cysteine (4).

Perhaps cystamine or its decomposition products show in the medium either a direct effect, as on prophage during induction of lysogenic cells and on DNA in the case of infection of sensitive cells or it acts on a repressor system which blocks prophage, as a result of which the possible synthesis of phage (γ) genetic material is inhibited.

However, both a direct connection of cystamine with prophage and an indirect one (with the repressor), if they exist, are unstable, which is indicated by the significant increase in the number of induced bacteria when irradiation is carried out 240 min after addition of the preparation. In addition, the role of oxidation of the preparation in the decrease in effectiveness is not excluded.

It is certain that the action of cystamine in induced phage production is a complex mechanism, the development of which depends greatly on the conditions of the experiment.

We noted the discrepancy between the decrease in the number of induced bacteria and the number of colonies after irradiation which may be explained by the lysing of some bacteria without the production of phage as a result of the effect of cystamine on prophage or repressors, and also by the lysing of bacteria with the production of defective phages. It is possible also that phages develop in the bacteria but do not mature. The solution of this problem is linked with an ultrastructural analysis of bacterial sections.

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