A CHLORAMPHENICOL RESISTANT HOST PROTEIN INVOLVED IN LYSOGENIZATION

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Evidence from genetic[13,14] and biophysical[15] studies indicates that in λ infection viral DNA replication precedes integration of λ genome into the host chromosome. We observed that λ sensitive strains of Escherichia coli K12 treated with 100 µg/ml of chloramphenicol (CM) inhibited the early infective processes leading to lysogenization by coliphage λ. CM pre-treated cells when transferred to CM free medium showed nearly 50 per cent increase in the frequency of lysogenization in the strain U279 (Str^S) during 90 minutes of growth, but there is no indication of such an increase in strain C600 (Str^r) under similar conditions.

Viruses show different degrees of genetic autonomy as far as the replication of their DNA is concerned. Larger viruses with more genetic material and information require from the host only a supply of deoxy-nucleotide triphosphate precursors[1]. Simpler viruses, on the other hand, may rely not only on precursors, but also on host polymerases and even host sites for DNA replication. A chloramphenicol resistant host protein has been demonstrated to be necessary for initiation of viral DNA replication in S13[2], ØX174[3] and λ[4] infections. This protein continues to be made in 30 µg/ml of chloramphenicol, but not appreciably in presence of 100 µg/ml[4]. Such a chloramphenicol-resistant virus-directed protein, associated with the synthesis of progeny RF (replicative form) has been isolated in cells infected with ØX174[5]. These types of proteins are suspected to be initiator proteins. New deoxyribonucleases have been demonstrated in infections with T-even phages[6-8], T5[9], λ[10] and poxvirus[11]. The functions of these enzymes in the infective process are not clearly understood. It now appears that both an exonuclease[10] and probably an endonuclease[12] are synthesised after λ infection which are controlled by the virus genome.

Taking frequency of lysogenization as an index for the initiation of virus multiplication (intracellular), we studied the ability of Escherichia coli K12 strains to lysogenize wild type λ (λ^+) at various stages of treatment of cells with chloramphenicol (CM). The results indicated a progressive decrease in lysogenization (60 to 70 per cent) following CM treatment (100 µg/ml) of exponentially grown cultures. When CM and λ were added

simultaneously lysogenization proceeded normally, but when CM treatment preceded λ infection, frequency of lysogenization was much reduced. Thus it appears that CM somehow inhibits the initiation of viral infective processes early in the intracellular stages of growth. The mechanism of this inhibition is still obscure.

Materials

Bacterial strains: strains of Escherichia coli K12, C600 (λ^s str^r leu thr lac su^+) and U279 (λ^s str^s gal try su^-) were obtained from the stocks of Enrico Calef[16].

Phage strain: wild type $\lambda(\lambda^+)$ was obtained from Enrico Calef[16].

Methods

In general, the methods described by Adams[17] were followed. The bacterial strains were grown at 37° in tryptone broth[14] with aeration to about 2×10^8 cells/ml. Phage infections of bacteria were carried out in the tryptone broth suspensions at a multiplicity of 2-3 phage per bacterium. Chloramphenicol (CM) at 100 µg/ml was added at desired time to exponentially grown (2 hrs at 37°) bacterial cultures. Viable cells were scored in tryptone agar plates (1.5% agar); phage adsorption was determined by centrifugation of adsorption mixture; the unadsorbed phage scored on C600. Frequency of lysogenization was calculated from surviving bacteria by replicating overnight grown dilution plates on a lawn of C600 and inducing them with UV.

Results

Preliminary results of our experiments of λ infection of chloramphenicol treated cultures of C600 and U279 are represented in Fig. 1. The number of viable cells recovered after treatment with CM during 120 minutes of growth at 37° indicated that strain C600 (Str^r) was relatively more resistant than U279. The precentage of phage adsorbed during this period showed a distinct correlation with the surviving bacterial population. This was presumed to indicate that presence of CM in the adsorption mixture did not affect phage adsorption. Control experiments also showed that CM had very little effect, if any, on the viability of phage λ in liquid suspension.

After 15 minutes of preadsorption of λ^+ the cells were centrifuged and survivors scored on tryptone agar plates at suitable dilutions. The frequency of lysogenization was calculated from the surviving bacterial population. Control experiments showed that about 70-75 per cent cells of the input bacteria died during exposure to λ infection (at multiplicity of 3); lysogenization was thus calculated from the 25-30 per cent surviving population. It was noted that the number of cells lysogenic to λ^+ was inversely proportional to the time the cells were in contact with CM. Thus,

from 30 minutes onwards frequency of lysogenization fell steadily during the 120 minutes of experimentation. This was equally true for both strains C600 and U279, though C600 was more sensitive to CM treatment than U279 in this respect. But for the first 20-30 minutes the presence of CM did not alter the frequency of lysogenization as compared to the controls (without CM) which was above 90 per cent at all times. This was thought to indicate that (1) CM did not affect phage adsorption to viable cells, and that (2) simultaneous addition of phage and CM to cell suspensions in tryptone broth did not prevent λ from undergoing its early infective processes leading to lysogenization.

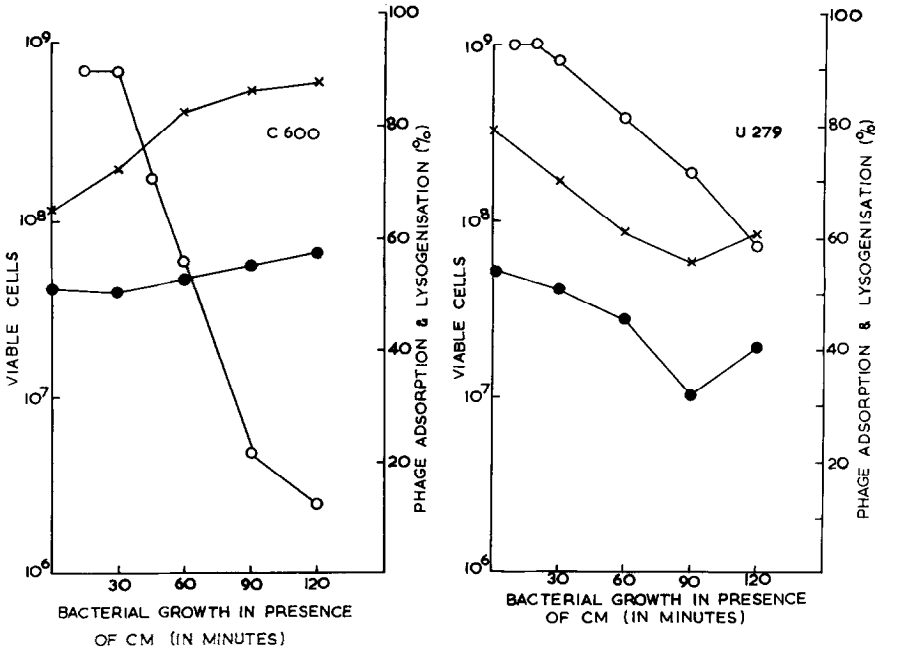

Fig. 1. Results showing differences in cell viability (●), phage adsorption (X) and frequency of lysogenization (O) in strains of C600 and U279 during treatment with 100 µg/ml chloramphenicol (CM) in tryptone broth during 120 minutes of experiments. Bacterial cultures were grown to exponential phase, centrifuged and resuspended in tryptone broth in presence of CM. Aliquots of CM treated cells were infected with λ^+ at a multiplicity of about 3.

A second set of experiments were performed to study the availability of phage specific proteins in CM pretreated cells. For this cells were grown in presence of 100 µg/ml of CM for about 120 minutes at 37°, centrifuged and resuspended in the presence (100 µg/ml) and absence of CM;

lysogenization was studied as before. Aliquots of cell cultures were infected with λ^+ at a multiplicity of about 2, at times 30 and 90 minutes of aeration. The results are presented in Table 1.

Table 1

Effect of chloramphenicol pretreatment on frequency of lysogenization by wild type phage λ^+

Bacterial strains	Frequency of lysogenization (in percentage)			
	30 minutes		90 minutes	
	-CM	+CM	-CM	+CM
C600	10.0	10.6	12.0	11.8
U279	82.5	45.0	98.2	50.4

The two strains, C600 and U279, responded very differently as regards availability of phage specific proteins for λ replication and lysogenization. Increase in frequency of lysogenization when CM pretreated cells were grown in absence of CM was considered to indicate that the host proteins needed for vegetative multiplication of virus prior to lysogenization was available to newly infecting virus particles. It was observed that biosynthesis of phage specific proteins was negligible in CM pretreated cells of C600, even when these cells were transferred to CM-free medium, so that λ^+ failed to lysogenize these cells even after 90 minutes of growth in CM-free medium (tryptone broth); strain U279, on the other hand, showed nearly 50 per cent increase in the availability of phage specific proteins under similar conditions as determined from an increase in frequency of lysogenization. Difference in the behaviour of these two λ^+ sensitive strains in this respect could not be explained from our experiments. Genetically, strain C600 is known to be different from U279. Strain C600 is an auxotroph (*leu thr lac*) and carries an amber suppressor (su^+) mutation, while strain U279 is known to carry *try gal* mutation, but does not have a suppressor mutation (su^-). Sensitivity of these two strains to CM (Fig. 1) treatment was also noted to be different. Whether streptomycin resistance of C600 could indirectly confer certain degree of cross resistance to chloramphenicol is not known. The only visible difference between these two bacterial strains is in the reinitiation of the biosynthesis of phage specific proteins by CM pretreated cells in CM-free medium. It is also possible that CM might bind irreversibly with certain host proteins in C600, thus making these proteins unavailable for λ development, whereas in U279 this CM-protein complex was relatively less stable in CM-free medium . Host specified conditions for lysogenization

were thus reinstated in the latter case. The possibility of some ribosomal participation in the case of the streptomycin resistant strain C600 in blocking virus multiplication could not be ruled out.

Discussion

Addition of chloramphenicol (CM) to exponentially growing cultures of Escherichia coli K12 can reduce the rate of protein synthesis by more than a hundred fold[18]; but initially, RNA synthesis is scarcely affected by the drug, and RNA content per cell approximately doubles in 90 minutes of CM treatment. Much of the RNA synthesised by the drug is found associated with protein in particles that sediment at 18 to 25s and are called chloramphenicol particles[18]. RNA extracted from these particles sediments at 16s to 23s, like the RNA extracted from mature ribosomes. It was thought[19], therefore, that the protein associated with CM particles was drawn from a pool of free ribosomal protein existing in the cell at the time of addition of chloramphenicol. But subsequent experiments[20] showed that the majority of the protein of CM particles was probably not ribosomal nor ribosomal precursors, but soluble protein present in the cell at the time of addition of the drug.

Thus, the results of our experiments described here would indicate that certain soluble proteins in E. coli K12 cells are either directly or indirectly involved in the early stages of λ infection leading to lysogenization. Whether the proteins involved are virus coded or act as initiators, we do not know. We could only assume that (1) chloramphenicol inactivates certain soluble proteins rendering these proteins unavailable to the virus for multiplication and for lysogenization, and that (2) different bacterial strains could show different degrees of capability to reinitiate the biosynthesis of phage specific proteins or in the stability of the CM-protein complex when transferred back to CM-free medium. It might be of interest to mention in this respect that U279 (su^-) always exhibits higher efficiency of plating of wild type λ than C600 (su^+) by nearly a factor of 2 (unpublished results). This might indicate that the infective processes in the intracellular stages of λ development are for some reason more efficient in U279 than in C600. Whether this has anything to do with the more efficient reinitiation of protein biosynthesis (viral) in U279, as observed in our experiments, is difficult to comprehend.

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