TRANSITION FROM STREPTOMYCIN-SENSITIVE TO STREPTOMYCIN-RESISTANT PROTEIN SYNTHESIS DURING BACTERIOPHAGE T4 DEVELOPMENT

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Summary:

Streptomycin completely arrests bacteriophage T4 development in streptomycin-sensitive Escherichia coli strain B when added immediately after phage adsorption. Under these conditions, streptomycin inhibits protein synthesis in E.coli multiply infected with bacteriophage T4 to the same extent as in exponentially growing uninfected cells. In the course of bacteriophage infection, a gradual transition occurs from streptomycin-sensitive to streptomycin-resistant protein synthesis. This process is accompanied by a decreased binding of streptomycin to ribosomes so that the ribosomes become protected against the effect that streptomycin exerts on the stability of the initiation complex. When streptomycin is added 8 to 9 minutes after infection, the overall rate of protein synthesis is no longer affected by the antibiotic, and the cells are now capable of supporting an almost normal phage development.

Introduction.

In a previous communication from this laboratory we reported that cyclic AMP enhanced the bactericidal effect of low to moderate concentrations of streptomycin (1). In bacteria, cyclic AMP stimulates the synthesis of messenger RNA of several inducible enzymes. Our results suggested, therefore, that streptomycin lethality was linked to streptomycin-induced synthesis of a specific messenger RNA, the synthesis of which was stimulated by cyclic AMP, and which coded for a bactericidal protein.

It appeared, therefore, of interest to study the effect of streptomycin on E.coli under conditions where the synthesis of bacterial messenger RNA was effectively shut off. Such a system is represented by cells infected with T-even bacteriophages. We were encouraged in this respect by the finding of Freda and Cohen (2) who reported that streptomycin at moderate concentrations did not very sharply affect the multiplication of T6r^{+} phage in streptomycin-sensitive THU and TAU mutants of E.coli reducing the yield of phage about 60 to 75%.

By using streptomycin at concentrations that inhibited protein synthesis and killed uninfected bacteria within 5 to 15 minutes of exposure to the drug, we made the observation that during phage development there occurred a shift from strepto-

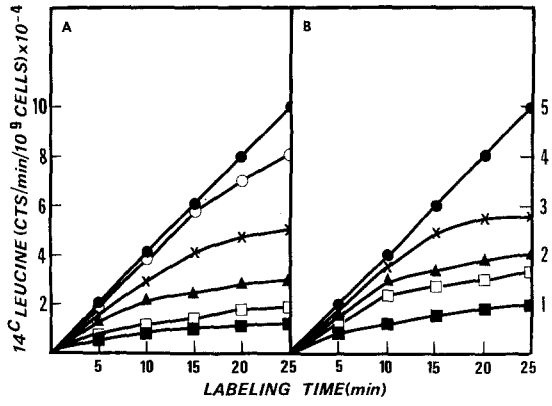

Fig.1.Effect of streptomycin on the incorporation of radioactive leucine in uninfected and T4-infected ,streptomycin-sensitive E.coli B.Cells were grown exponentially to $3x10^8$ cells/ml in Fraser-Jerrell medium at 37°C,concentrated by centrifugation to 10^9 cells/ml in the same medium containing 0.12 g of casamino acids per 1000 ml, and l-tryptophan,100 ug/ml.Part of the cells was infected with bacteriophage T4 at m.o.i.of 7.After 2 minutes of adsorption,free phage particles were removed by filtration and all cultures were diluted to $3x10^8$ cells/ml in fresh medium.At this point radioactive label and streptomycin were added.Samples were processed as described in the Materials and Methods section.A - uninfected cells; B - infected cells; No streptomycin added, ●—● ; Streptomycin,20 µg/ml, O—O ; Streptomycin, 50 µg/ml, x--x ; Streptomycin,100 µg/ml, ▲--▲ ; Streptomycin,200 µg/ml □—□ ; Streptomycin, 500 µg/ml, ■—■ .

mycin-sensitive to streptomycin-resistant protein synthesis.This observation is the subject of the present communication.

Materials and Methods.

Bacteria and phage.Escherichia coli strains used were B,sensitive and resistant to streptomycin.The phage strain used was T4D.Phage stocks were prepared by fluid lysis in broth,purified and stored as described by Herriot and Barlow (3).

Infected cells.Bacteria were grown at 37°C with agitation to $3x10^8$ cells/ml in the medium of Fraser and Jerrell (4) with a generation time of 35 minutes.Phage adsorption,bacterial survival,the number of infective centers,and one -step growth curves were determined by standard techniques(5).Intracellular phages were assayed by the lysozyme-EDTA-SDS method (6).At the end of adsorption period more than 99.5% of the bacteria were killed.More than 95% of the killed bacteria could be accounted for as infective centers.Under the specified conditions,the ecclipse period was 15-19 minutes,and a maximum phage yield was obtained 30 minutes after infection.

Incorporation of radioactive leucine was measured as previously described (7). 70-S ribosomes were prepared by the method of Nirenberg and Matthaei (8).Streptomycin binding to ribosomes was assayed by the procedure described by Biswas and

Gorini(9) and the T4-mRNA dependent binding of fMet-tRNA to 70-S ribosomes was measured by the method of Lelong et al.,(10).Pulse-labeled T4-mRNA was prepared as described (11).

Chemicals. ^{14}C-streptomycin chloride was a generous gift of Merck & Co.,Rahway,N.J. 2-^{14}C-leucine (54 mCi/mmole) and ^{3}H-uridine (22 C/mmole) were purchased from Schwarz/Mann,Orangeburg,N.Y.Lysozyme and streptomycin sulfate were obtained from Calbiochem, Los Angeles,Calif.Anti-T4 serum was procured from Midwest Culture Service,Terre Haute, Imd.All chemicals were of analytical grade.

Results.

Streptomycin acts as an antiviral agent for certain bacterial viruses since it is inhibitory to the phages even when the host bacteria are resistant to the antibiotic (12).Of E.coli phages apparently only RNA phages are sensitive to streptomycin.Bacteriophage T4 used in this study is not affected by streptomycin since E.coli B resistant to streptomycin can support normal bacteriophage growth even in the presence of 1000 ug of streptomycin/ml.

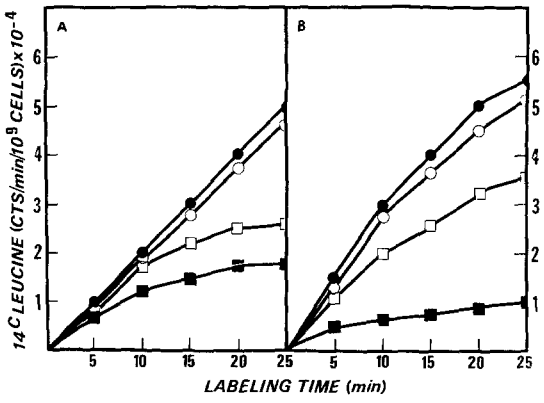

Fig.2.Transition from streptomycin-sensitive to streptomycin-resistant protein synthesis during phage T4 development.For experimental conditions,see Fig.1.
A - streptomycin,100 ug/ml; B - streptomycin,500 ug/ml.No streptomycin added,●—● ; streptomycin added 2 min.after adsorption,▲—▲; streptomycin added 5 min.after adsorption, □—□ ; streptomycin added 8 to 9 min.after adsorption, O—O .

Fig.1 shows the effect of streptomycin on protein synthesis by uninfected and phage T4-infected streptomycin-sensitive E.coli.After T4 infection,the overall rate of protein synthesis decreased to about 50% of the pre-infection level.This observation was first made by Gau sing (13),who showed that the decrease in the rate of protein synthesis after phage infection was mainly due to the reduction in the growth rate of peptide chains.Streptomycin inhibited protein synthesis in both the uninfected and bacteriophage T4-infected cells.

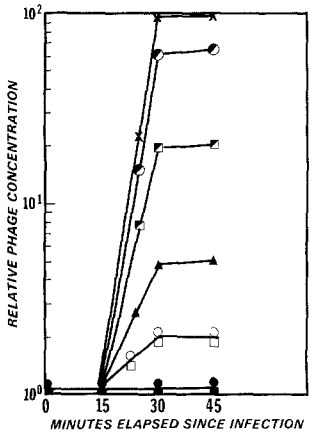

Fig.3.One-step growth of bacteriophage T4 in streptomycin-sensitive E.coli B. Bacteria at a density of 10^9 cells/ml were infected with phage T4 at m.o.i. of 7,incubated for 2 minutes to allow fixation to bacteria of the phage particles and diluted 40 fold into an appropriate concentration of anti-T4 serum,which neutralized all the remaining free phage particles within 2 minutes.The infected cells were then rapidly diluted 250 fold into fresh medium.At this point streptomycin was added to one portion of the infected cells.Other portions received streptomycin from 4 to 9 minutes after adsorption.All cultures were incubated at 37°C, and samples were taken every 15 minutes for intracellular phage assay.Infection in the absence of streptomycin ,x---x;Streptomycin,100 ug/ml:added 2 min.after adsorption,●---●; 4 min.after adsorption,○---○; 5 min.after adsorption,▲---▲; 8 min.after adsorption,◐---◐. Streptomycin,500 ug/ml.added 2 min.after adsorption, ■---■ ; 5 min.after adsorption, □---□ ; 9 min.after adsorption, ◧---◧ .

Fig.2 shows protein synthesis in phage T4-infected cells to which streptomycin was added at various time intervals during phage development.It can be seen from the figure that during the first 9 minutes of phage infection there occurs a transition from streptomycin-sensitive to streptomycin-resistant protein synthesis. Addition of streptomycin to E.coli 2 minutes after infection resulted in complete arrest of protein synthesis within 5 to 10 minutes of exposure to the drug.Streptomycin added 4 to 6 minutes after infection was much less inhibitory.When streptomycin was added 8 to 9 minutes after infection,the rate of protein synthesis remained unaffected by streptomycin through the completion of the lytic cycle,and was the same as in control cultures not exposed to the antibiotic.

These experiments were repeated many times.The results were reproducible in the sense that protein synthesis was always more resistant to streptomycin when the drug was added later in infection.In some experiments,the resistance of protein synthesis to high concentrations of streptomycin added 8 to 9 minutes after infection was not absolute.The cells continued to incorporate the label at an undiminished rate for about 15 minutes following the addition of the drug.Thereafter,

Table 1. Binding of streptomycin to 70-S ribosomes extracted from uninfected and phage T4-infected, streptomycin-sensitive E.coli.

Exp.	Streptomycin present during binding reaction (conc.)	Molecules of streptomycin bound per 70-S ribosome: before infection	2 min. after adsorption	8 min. after adsorption	10 min. after adsorption
I	10^{-5}M	1.00	1.00	0.50	0.30
II	1.5×10^{-5}M	1.30	1.35	0.60	0.40
III	10^{-5}M	1.10	1.00	0.50	0.35
IV	2×10^{-5}M	1.30	1.10	0.60	0.30

The binding mixture contained: 70-S ribosomes, 4.3×10^{-6}M (or 3×10^{-5}M); streptomycin, 0.052 uCi/mg, 10^{-5}M to 2×10^{-5}M; Tris-acetate buffer, 0.01M, pH 7.8; KCl, 0.06M; and magnesium acetate, 0.01M; in a total volume of 1 ml. The mixture was incubated at 37°C for 15 min. After incubation, the mixture was dialyzed at 4°C against 20 volumes of the same buffer with four changes of buffer. 0.2 ml samples of the dialyzed mixture were dissolved in NCS and counted in Liquefluor. A 1:1 molar ratio of streptomycin: :70-S ribosome is equivalent to 0.705 ug of streptomycin per 3 mg of 70-S ribosomes. Under our experimental conditions, 1 ug of radioactive streptomycin is equivalent to 105 counts per minute above backrgound of 25 to 39 counts per minute.

the rate of protein synthesis decreased by about 20 to 25% of the control, and the cells continued to synthesize protein at this lower rate until the end of the lytic cycle.

We next studied the effect of streptomycin on the ability of E.coli to support phage growth. The results of a typical experiment are illustrated in Fig. 3. It can be seen from the one-step growth curves that the addition of streptomycin 2 minutes after phage adsorption completely inhibited intracellular phage formation. Phage development was also drastically inhibited in cultures to which streptomycin was added 4 and 5 minutes after adsorption. Addition of streptomycin 8 to 9 minutes after adsorption allowed the cells to support an almost normal phage development. The yield of infective phage particles that could be released from cells incubated in the presence of 100 and 500 ug of streptomycin/ml was 70 and 25% of the control, respectively. In numerous experiments of this kind, the yield of infective phage particles from cells incubated with 100 and 500 ug of streptomycin per ml ranged from 50 to 88 and from 25 to 55% of the control, respectively.

The following experiments indicate that the transition to streptomycin-resistant protein synthesis may be due to the acquisition of streptomycin resistance by host ribosomes.

The data presented in Table 1 show that the transition to streptomycin-resistant protein synthesis during phage T4 development was accompanied by a marked decrease

Table 2. The effect of streptomycin on T4 mRNA-dependent binding of fMet-tRNA to 70-S ribosomes from uninfected and T4-infected E.coli B.

Ribosomes extracted from	T4 mRNA-dependent binding of f/^{3}H/Met-tRNA		
	total		inhibited by streptomycin(%)
	- streptomycin	+ streptomycin	
E.coli B before infection	750	155	79.0
E.coli B infected with T4 for 2 min.	680	170	75.0
E.coli B infected with T4 for 10 min.	675	600	11.0

The reaction mixture contained in 50 ul:50 mM Tris-HCl,pH 7.5;8 mM magnesium acetate; 80 mM NH_4Cl; 7 mM 2-mercaptoethanol; 1 mM GTP; 70 ug late T4 mRNA; 40 pmol f/^{3}H/Met-tRNA, (about 700 counts/min/pmol); 8 ug streptomycin,when present;and 40 ug 70-S ribosomes. Backgrounds without T4 mRNA were subtracted from each value.The binding was measured by the millipore filtration technique(10).

in the ability of E.coli ribosomes to bind the drug.Under the specified conditions, ribosomes from uninfected cultures and from cells infected with phage T4 for 2 minutes bound approximately one molecule of streptomycin per 70-S ribosome.The ability of host ribosomes to bind streptomycin was reduced to 0.5 and 0.3 molecules of the antibiotic per 70-S ribosome after 8 and 10 minutes of phage development,respectively.

In the line with the above results on ^{14}C-streptomycin binding it was found that ribosomes isolated from cells infected with phage T4 for 10 minutes were protected against the effect that streptomycin exerts on the stability of the initiation complex.As shown in Table 2,the T4 mRNA-directed binding of fMet-tRNA is affected by streptomycin.The amount of bound initiator tRNA to ribosomes isolated from streptomycin-sensitive E.coli B prior to infection or shortly after infection with phage T4 was reduced by streptomycin by about 80%.When ribosomes were extracted from cultures of E.coli after 10 minutes of infection with phage T4,streptomycin-induced inhibition of fMet-tRNA binding was almost totally suppressed.

Discussion.

The finding that the ability of E.coli ribosomes to bind streptomycin decreased during phage T4 development and that ribosomes extracted from cells infected with the phage were protected against the action of streptomycin on the initiation complex suggested that modification of host ribosomes may be responsible for the tran-

sition to streptomycin-resistant protein synthesis observed after infection with phage T4.

After infection of E.coli by phage T4,modification of host ribosomes was observed,which restricted the translation of host and other RNAs unrelated to T4(14,15, 16).Several new proteins were found in the ribosomal factor fractions and a major one associated with salt-washed ribosomes,presumably a ribosomal structural protein (14).The latter was not apparent until 10 minutes after infection.The relevance of these proteins to the functional alterations of ribosomes is not yet clear,but it would appear that at least the newly synthesized or modified factor B (F3) may be responsible for the messenger selection process (16).In in vitro experiments, cooperative binding of F1,F2 and F3 factors to ribosomes extracted from uninfected E.coli protected ribosomes against streptomycin(10).Thus,both the P10 ribosomal protein and initiation factors appear to be involved in streptomycin binding, and both may affect the response of ribosomes to the antibiotic.

It seems probable that the phage-specific proteins confer streptomycin resistance on the ribosomes during phage T4 infection.Modification or substitution of ribosomal factors and/or ribosomal structural protein (especially if the latter proves to be a P10-like protein involved in chain initiation)may be the device used by virulent phages to turn the host's protein synthesizing machinery to their advantage.The fact that the modified ribosome becomes resistant to streptomycin is merely a coincidence. The problem whether it is the ribosomal structural protein or ribosomal factor(s) or both that confer streptomycin resistance on the ribosomes during phage infection is under investigation.

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