EFFECT OF STREPTOMYCIN ON BACTERIOPHAGE ϕX174

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The inhibitory action of certain cationic and anionic agents on the T2 phage-antiphage system has been reported (Mora and Young, 1962). During the course of testing for the inhibitory action of certain of these agents on the neutralization reaction of bacteriophage ϕX174, it was noted that streptomycin sulfate (SM) enhanced the neutralization reaction rather than inhibiting it, while not affecting the ϕX174 virions in the absence of antibody. Brock (1962) also showed that SM has no effect on bacteriophage ϕX174, but that it does inactivate streptococcal bacteriophage P9 apparently by preventing injection of DNA by cross-linking between adjacent protein subunits (Brock et al., 1965). This paper presents preliminary studies concerning the effect of SM on the ϕX174 phage-antiphage system.

MATERIALS AND METHODS

The bacteriophage used was the ϕX174 preparation previously described by Bowman and Patnode (1964a) or a derivative of that preparation. Phage assays were made on host bacteria, Escherichia coli, strain C or C/SM, by the overlay agar technique (Adams, 1959). The specific antiserum was obtained from a single bleeding of a rabbit during its secondary response to purified ϕX174 virions. SM (sulfate salt) was obtained from Calbiochem, Los Angeles. Sterile demineralized water was employed in the reaction mixtures and as the diluting medium.

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RESULTS AND DISCUSSION

In all experiments, the addition of SM to the ϕX174 phage-antiphage system consistently resulted in decreased plaque counts relative to the control neutralization mixture containing no SM. This decrease ranged from two- to tenfold. The results of an experiment showing this effect are presented in Table I. It can be seen that there was approximately a 10-fold greater decrease in plaque formers in the reaction mixture containing phage, antibody, and SM than in the mixture containing phage and antibody.

TABLE I

Effect of SM on the ϕX174 phage-antiphage reaction

Mixture*	Plaque count/ml x 10^6	
	No incubation	After 40 min. at 37°C
ϕX174 alone	3.89	3.84
ϕX174 + SM**	3.44	3.12
ϕX174 + antibody***	--	0.69
ϕX174 + antibody + SM	--	0.07

*1 ml
**SM = 275 μg/ml
***Antibody diluted 1:1000

In support of Brock's work (1962) we found no significant difference in the number of plaques formed when phage ϕX174 is plated on agar with or without SM, (E. coli, C/SM, being used as plating bacteria in the latter case). In addition, if the phage was incubated with SM prior to plating on agar with or without SM, no significant differences were found. These results, seen in Table II, clearly indicate that in the absence of antiserum SM has no inhibitory effect on the phage.

We next tested for the reversal of the SM effect by magnesium ions (Mg) (Brock and Wooley, 1963). For this purpose, an experiment similar to that shown in Table I was performed except that Mg was

TABLE II

Effect of plating mixtures of phage ϕX174 on medium with and without SM*

Reaction mixture**	Time of incubation*** (min)	Plaque count/ml (x 10^5)	
		Agar without SM	Agar with SM
ϕX174 alone	0	2.89	3.03
	40	2.87	2.67
ϕX174 + SM*	0	3.26	2.85
	40	2.73	2.55

*250 μg/ml
**1 ml
***37°C

added to various mixtures. Table III shows the results and comparing mixtures 4 and 7, it can be seen that Mg did not reverse the effect of SM. The results also show that the activity of the antiserum was greater in the presence than in the absence of Mg (compare mixtures 3 and 6). At present it is not known if the increase in activity of the antiserum on phage ϕX174 by SM and Mg is due to the same or different mechanism(s). In this experiment no explanation is available to account for the failure to obtain a tenfold enhancing effect by SM on neutralization as was obtained in the experiment shown in Table I.

The results of the above experiments do not indicate whether the action of SM is on intact virus (the results of which would make the virus more sensitive to antibody) or on virus-antibody complex. Therefore, a series of experiments were performed to determine the effect of sequential treatment of the virions, first with specific antibody followed by SM, or vice versa, on the number of phage survivors. In these experiments phage ϕX174 was incubated for 30 minutes with one of the reagents (antiserum or SM) and then diluted 1:10 into the other reagent and further incubated for 30 minutes. Assays for

TABLE III

Effect of $MgSO_4$ on the neutralization of phage ϕX174

Phage mixed with:	Plaque count/ml x 10^8	
	No incubation	After 40 min at 37°C
1. HOH*	1.16	1.02
2. SM**	-	1.04
3. Antibody***	-	0.091
4. Antibody + SM	-	0.059
5. SM + Mg****	-	0.78
6. Antibody + Mg	-	0.037
7. Antibody + Mg + SM	-	0.041

*1 ml volume in all mixtures
**250 μg/ml
***Diluted 1:1000
****10^{-2} M

surviving phage were made at each of these time intervals. Figure 1 gives the procedure and results of one of these experiments. It can be seen that the number of plaque formers remained constant during the first 30 minutes of incubation of the virus in the absence or presence of SM (curves A and B, respectively). The results of treating the phage with antibody first (neutralization) is shown in the upper part of curve C. At the 30 minute point in this experiment, the neutralization mixture was diluted 1:10 into SM and 1:10 into water and incubated. As shown (curve C) the incubation with SM, but not with water, resulted in a further decrease (about 10-fold) in plaque formers. Diluting the control reaction mixture, containing only phage, 1:10 into SM or water did not result in a significant change of plaque formers; however, if diluted 1:10 into antiserum, normal neutralization occurred (curve A). Diluting the control mixture, containing virus and SM, 1:10 into water caused no significant decrease in the plaque formers; however, diluting it 1:10 into antiserum

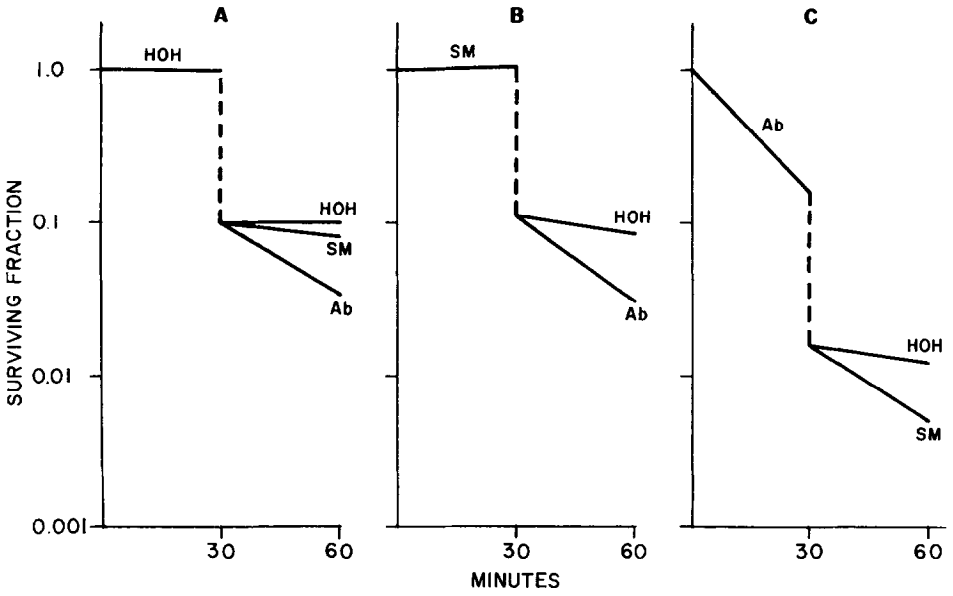

Figure 1. Effect of sequentially treating phage ϕX174 with specific antiserum (Ab) followed by streptomycin (SM). Input phage = 7.55 x 10^7 plaque-formers/ml for each experiment. SM = 250μg/ml. Ab = diluted 1:1000. The phage was incubated (37°C - 30 minutes) with: (A) HOH, then diluted 1:10 into HOH, SM, and Ab then incubated; (B) SM, then diluted 1:10 into HOH and Ab then incubated; (C) Ab, then diluted 1:10 into HOH and SM. Dotted vertical lines represents various 1:10 dilutions.

resulted in normal neutralization (curve B). Comparing curves B and C, it can be seen that sequentially treating the phage in the order, antiserum-SM, gave approximately ten times more inactivation of phage than the SM-antiserum sequence of treatment. It therefore appears that SM is active on the virions only after their reaction with specific antibody.

SUMMARY AND CONCLUSIONS

Streptomycin (SM) has no effect on the replication of phage in plating experiments or on aqueous suspensions of the phage. However, in aqueous suspensions of the phage containing specific antibody and SM, greater inactivation occurs than with antiserum alone. Magnesium sulfate (10^{-2} M) does not reverse the enhancing effect of SM on the

ϕX174 phage-antiphage reaction. Antiserum has a greater effect on the phage in the presence of magnesium ions than in their absence. Sequential treatment of the virions, first with specific antibody and second with SM, inactivates more phage (approximately 10-fold) than does treatment with SM followed by specific antibody.

The following mechanism (others are possible) is presented as being consistent with the results. In view of the fact that SM is thought to complex with DNA (Brock and Wooley, 1963) and since SM apparently has no effect on ϕX174 coat protein (Table II and Brock, 1962), it is suggested that the reaction between antibody and ϕX174 virions results in an altered configuration without neutralization of the virus particle with which SM can react and exert an inhibitory effect, presumably combining with accessible phage DNA. Previous work by Bowman and Patnode (1964 a and b) and Rolfe and Sinsheimer (1965) supports this hypothesis.

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