INTERACTION OF BASIC ANTIBIOTICS WITH PHAGE f_2 PARTICLES

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Received October 30, 1964

It was recently shown by Brock (1962) that streptomycin inhibits reproduction of MS2 phage in a streptomycin-resistant host. Moreover, it was shown that streptomycin (stm) interferes with the reproduction of phages μ_2, f_2 and f_d respectively, in streptomycin-resistant hosts and that it inhibits reproduction of phage f_2 in stm-sensitive host cells treated with five minutes pulse of 2 µg/ml of stm during adsorption period, i.e. under conditions which do not affect the host cell (Schindler 1964).

Stm has an affinity towards some anionic macromolecules. With DNA, RNA, heparin, dextran sulfate etc., it forms precipitating complexes (Gros et al. 1949, Rybak, Gros, Grumbach 1949, Berkman, Housewright, Henry 1949, Hoffman et al. 1959, Cohen and Lichtenstein 1960, Moskowitz 1963). Stm is capable of binding to ribosomes (Willick and Polglase 1963) and to some bacteriophages (Cohen 1947, Aronson, Meyer, Brock 1964) as well.

Interaction of stm, neomycin and kanamycin with free phage particles was studied in order to determine its possible role in the inhibition of reproduction of f_2 phage by these antibiotics.

MATERIAL AND METHODS

The bacterial strains (E.coli K 12 R1, E.coli K 13),

f_2 phage strain, media and general techniques were described in a previous paper (Schindler 1964). A neomycin-resistant mutant was obtained by means of UV irradiation of a suspension of E.coli K 13. Titration of inactivated lysates was not influenced by stm or neomycin, since both were diluted to 0,01 µg/ml and more. These concentrations do not interfere with plaque formation.

Streptomycin sulfate Specia, Neomycin sulfate Lundbeck, Kanamycin sulfate, heparin Novo, DNA Light, dextran sulfate DS 2000 Pharmacia and dextran sulfate DS 60 (kindly furnished by Dr.J.Málek, ÚSOL, Prague) were used in our experiments. Ribonucleic acid from yeast was prepared according the method of Crestfield, Smith and Allen (1962, in Allen 1962).

Bacteriophage f_2 was partially purified with ammonium sulfate, RNAase and by centrifugation 2 hrs at 100,000 G (Loeb and Zinder, 1961).[x/]

Agar-gel electrophoresis of partially purified phage was kindly performed by Dr.John and PhMr.Dušková. It was run in veronal buffer, pH 8.2 for 3 hrs at 6V/cm. The agar layer was then cut into 5 mm strips which were eluted in 5 ml 0,5 % peptone water overnight at 37 C. The eluate was titrated.

RESULTS

Inactivation of phage f_2 by stm, neomycin and kanamycin. After adding stm or neomycin in concentrations up to 1000 µg/ml to a diluted lysate (3.7 . 10^8 pfu/ml) and incubating at 37 C for 60 minutes a drop in titer could be observed. The degree of inactivation depends on antibiotic concentration as shown in table 1. Stm exerts a higher inactivating effect

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[x/] The help of Ing.Šípalová, Institute of Haematology, Prague, is gladly appreciated.

than the same concentration of neomycin. Kanamycin is ineffective even in a concentration of 1000 ug/ml.

Table 1 Inactivation of phage particles by stm, neomycin and kanamycin

U µg/ml	10^8pfu/ml	%	10^8pfu/ml	%	10^8pfu/ml	%
0	3.7	100	3.7	100	3.7	100
10	2.8	76	3.3	89	2.2	84
100	2.3	62	3.4	92	3.1	118
1000	0.12	3.4	2.2	59	2.5	99
10000	0.07	1.9	1.7	46	3.7	147
	streptomycin		neomycin		kanamycin	

Phage lysate was diluted to a concentration of the order 10^8 pfu/ml. Respective antibiotics were added in appropriate concentrations. Lysate was titrated after 60 min incubation at 37 C.

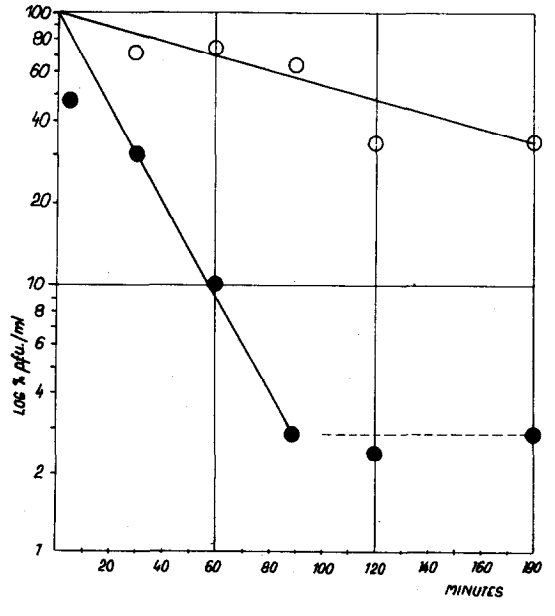

Fig.1. Kinetics of inactivation of f_2 by stm ● and neomycin ○ .

Inactivation of f_2 phage by stm and neomycin follows first order kinetics / Fig.1 /. Velocity constant of inactivation (37 C, 1000 ug/ml) for stm and neomycin being K= 0,038 min^{-1} and K= 0,0065 min^{-1} respectively. The reproduction-inhibiting effect of these two antibiotics is quite reversed (Fig.2).

Neomycin has higher reproduction-inhibiting effect than stm, being active even in concentrations at which stm does not exert any inactivating effect on free phage.

Inhibition of stm-inactivation of phage f_2.

By treating free phage with stm and heparin, dextran sulfate, DNA and RNA respectively, a diminution of inactivation can be observed. Dextran sulfate inhibits inactivation by stm even when added 30 min after stm. Similar effect is exerted by Mg^{++}. If stm is preincubated in 0,1 M $MgCl_2$ and then incubated with phage lysate, no inactivation can be observed (Tab.3). Since inactivation by stm can be inhibited by Mg^{++}, we assumed that complexes of stm and phage might be uncoupled by Mg^{++} too. Lysate which was inactivated up to 99,4 % by stm, could be reactivated by diluting in saline or 0,5 % peptone, both containing 0,1 M $MgCl_2$ (Tab.3). In both instances following reactivation, titers were higher than the original titer of the lysate. This, perhaps, is due to the influence of Mg^{++} on penetration of nucleic acid into the cell (Brock and Woolley 1963).

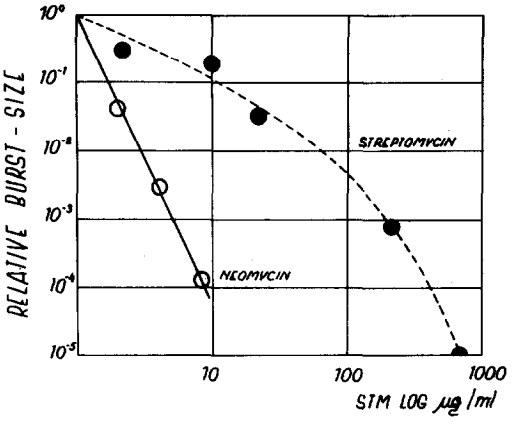

Fig.2. Inhibition of f_2 phage reproduction by stm and neomycin respectively in resistant host E.coli K 15 stm^r and K 15 neo^r.

Stm or neomycin was added in respective concentration to the host cells (5.10^7 cells/ml) together with f_2 (1 pfu/cell). After 5 min infected culture was centrifuged, cells resuspended in the same medium with antibiotic. Infective centres were determined. Average burst was expressed as pfu per infective centre. For relative burst calculation control was put equal 1.

Table 2. The effect of anions on inactivation of f_2 phage by stm

STM µg/ml	ANION	µg/ml	% pfu/ml	STM µg/ml	ANION	µg/ml	% pfu/ml
—	—		100	5000	DNK	5000	16
5000	—		1,1	—	DNK	5000	106
5000	heparin	1250	59	5000	RNK	5000	21.7
—	heparin	1250	108	—	RNK	5000	95.7
5000	DS 60	5000	50	5000	DS 2000	5000	15.7
—	DS 60	5000	115	—	DS 2000	5000	87

Phage lysate was diluted to the concentration of the order of 10^9 pfu/ml and stm or stm plus a respective anion was added (DS 60 = dextran sulfate DS 60, DS 2000 = dextran sulfate DS 2000 Pharmacia, Sweden). After 60 min incubation at 37 C, the lysate was titrated.

Reversibility of the binding of stm onto the phage particle was proved by means of electrophoresis in agar gel too. Partially purified phage did not exert any change in electrophoretic mobility after treatment with stm in concentrations 50 µg/ml, 100 µg/ml and 1000 µg/ml respectively. No inactivation was observed in the eluates of individual fractions after electrophoresis.

DISCUSSION

Both stm and neomycin form complexes with phage particles. The consequence of this binding is drop in titer of phage lysate. The binding is reversible and can be uncoupled by means of macroanions as well as by Mg^{++}. Both antibiotics bind by electrostatic forces probably to negatively charged groups of phage protein capsid. The interaction of stm or neomycin with phage f_2 is analogous to other systems, e.g. poly-l-lysine - T_2 phage (Shalitin, Danon and Katchalski 1962) or polycations - T_2 phage system (Mora and Young 1962).

Table 3. Uncoupling of streptomycin-phage complex by Mg^{++}

	pfu/ml	% pfu/ml
CONTROL	$1.7 \cdot 10^4$	100
control of inactivation by stm, without dilution	$1.1 \cdot 10^6$ x/	0.6
Inactivated lysate diluted in:		
saline	$2.8 \cdot 10^2$	2.4
saline + 0.1 M $MgCl_2$	$2.4 \cdot 10^4$	140
peptone	$8.7 \cdot 10^2$	4.9
peptone + 0.1 M $MgCl_2$	$4.1 \cdot 10^4$	236

x/ equivalent to $1.1 \cdot 10^2$ pfu/ml of diluted lysate

To the phage lysate, diluted to 10^8 pfu/ml, stm was added to the concentration 5000 ug/ml and incubated for 60 min at 37 C. Subsequently it was diluted 10^{-4} into four flasks which contained: 1. saline, 2. saline + 0.1 M $MgCl_2$, 3. 0.5 % peptone water, 4. 0.5 % peptone water + 0.1 M $MgCl_2$, respectively. After 120 min incubation at 37 C the lysate was titrated.

However, the inhibition of reproduction of phage f_2 cannot be explained only by the reaction of the antibiotics with the free phage. This seems to be confirmed by the fact that stm inhibits reproduction of f_2 at a concentration at which no inactivation occurs, as well as by the inversion of the ratio of inhibitory and inactivating activities of both stm and neomycin. Stm exerts a thousand-fold lower reproduction-inhibiting activity as against a twenty-fold higher inactivating activity of free phage than neomycin.

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