JOURNAL OF VIROLOGY, Oct. 1972, p. 869-871
Vol. 10, No. 4

Printed in U.S.A.

Effect of Zn^{2+} on the Adsorption of Male-Specific Filamentous Deoxyribonucleic Acid and Isometric Ribonucleic Acid Bacteriophages[1]

JONATHAN T. OU AND THOMAS F. ANDERSON
The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania 19111

Received for publication 10 May 1972

By means of both electron microscopy and plaque assay techniques, 10^{-3} M Zn^{2+} has been shown to reduce the adsorption rate of male-specific filamentous deoxyribonucleic acid bacteriophages to the tips of F pili. In contrast, 10^{-3} M Zn^{2+} did not affect the adsorption of male-specific ribonucleic acid phages to the sides of F pili.

Tzagoloff and Pratt (11) have reported that the attachment of the filamentous deoxyribonucleic acid (DNA) phages M13 to the tips of F pili can be inhibited by the addition of 10^{-3} M Zn^{2+}. We have repeated their experiment and, using the electron microscope, have also directly confirmed the interpretation of their results. However, the inhibition of attachment was not complete, furthermore, the DNA phages eventually overcame the inhibitory action of Zn^{2+} and infected the bacteria.

We have also found that 10^{-3} M Zn^{2+} has no detectable effect on the adsorption of ribonucleic acid (RNA) phages to the sides of F pili. This supports the suggestion that the site of action of the Zn^{2+} is at the tip of the F pilus (8).

The bacterial strains used and their relevant characteristics are as follows: strain HB11 of *Escherichia coli* B harbors F′ *lac*$^+$ and has F pili but no type I pili on its surface; strain HfrH of *E. coli* K12, a Hayes type Hfr, has both F pili and type I pili; and strain W1-3 of *E. coli* K12, an F$^-$, has type I pili.

The bacteriophages used are: f1, a male-specific filamentous DNA phage which adsorbs to the tips of F pili (2); R17 and MS2, male-specific isometric RNA phages which adsorb to the sides of F pili (3). These two RNA phages are closely related, and there is no difference in their adsorption behavior; therefore, they were used interchangeably.

Nutrient broth and nutrient agar plates were used for phage adsorption and plaque formation (8). Two milliliters of exponentially growing HB11 cells ($\backsim 4 \times 10^8$ cells/ml) were mixed with an equal volume of phage suspension (titer $\backsim 10^{12}$ phages/ml). For electron microscopy observation on the adsorption of f1 phage, RNA phages were added to identify the F pili. Ten minutes were allowed for phage adsorption, and 1% glutaraldehyde was added to fix the mixture of phages and bacteria (HB11). A drop of the fixed mixture was immediately placed on a 400-mesh grid which had been covered with thin carbon-coated Formvar; after 10 min the sample was washed several times with double-distilled water and stained with 2% uranyl acetate. The excess liquid was absorbed with filter paper, and the air-dried sample was examined in a Siemens Elmiskop I.

When a control mixture (no added Zn^{2+}) of bacteria and phages was examined in the electron microscope, attachment of f1 phages to the tips of F pili was readily seen; frequently two f1 phages were attached to one end of a single pilus. Unexpectedly, however, when the mixture with 10^{-3} M Zn^{2+} was examined carefully, the attachment of f1 phages to the tips of F pili was also seen, although with much lower frequency. It seems, therefore, that 10^{-3} M Zn^{2+} does not completely inhibit the adsorption of f1 phage.

To measure quantitatively the effect of 10^{-3} M Zn^{2+} on the adsorption of phages, random fields were observed, and every F pilus, both of whose ends could clearly be seen, was scored as either having or not having f1 phage attached. F pili having one end obscured under the metal of a grid were counted if the visible end had attached phage but were disregarded if no phage was attached, since it might have been the end of a broken pilus to which f1 phage could not have been attached (2). This procedure may bias both control and experimental results slightly in favor of the number of F pili with f1 phages attached to them.

As seen from the data listed in Table 1 as 2 × 2

[1] This work is dedicated to the memory of the late Jack Schultz.

TABLE 1. *Effect of 10^{-3} M Zn^{2+} on number of F pili with attached filamentous DNA phages as measured in the electron microscope*[a]

Determination	Control (no added Zn^{2+})	$+10^{-3}$M Zn^{2+}	Total
No. of F pili having no f1 phages attached	73	171	244
No. of F pili having one or more f1 phages attached	65	22	87
Total	138	193	331

[a] Chi-square, 57.16; degrees of freedom, $v = 1$; probability, $\ll 0.005$, calculated by the method indicated in reference 10.

contingency table, 47% of the F pili counted in the control mixture had attached f1 phages, whereas only 11.4% of the F pili had phage attached in the presence of 10^{-3} M Zn^{2+}. The chi-square test indicates that 10^{-3} M Zn^{2+} significantly reduces the adsorption of f1 phages to the tips of the F pili.

Similar counts were made of the absorption of the isometric RNA phage R17 to the sides of F pili. F′ HB11 bacteria ($\backsim 4 \times 10^8$ cells/ml) were mixed with equal volumes of R17 phage suspension containing 4×10^9 phages/ml. The mixtures were then prepared as described above. An arbitrary unit (about 1 μm) in length was established, and every attached R17 in the unit was scored. The average number of attched R17 was shown to be 9.4 particles per unit length for the control (without added Zn^{2+}) compared to 9.8 particles per unit length for the mixture containing 10^{-3} M Zn^{2+}. A statistical analysis using the Student's t test indicates that there is no significant difference between the two samples (Table 2).

Plaque assays were also used to determine the effect of 10^{-3} M Zn^{2+} on the adsorption of f1 and R17 phages. Exponentially growing HfrH cells ($\backsim 4 \times 10^8$ cells/ml) were mixed with an equal volume of low-titer phage preparation ($\backsim 10^8$ phages/ml for DNA phages and $\backsim 10^9$ phages/ml for RNA phages). After 10 min had been allowed for adsorption at 37 C, the mixture of bacteria and phages was centrifuged in the cold for 5 min at 9,000 $\times$ g. The titer of unadsorbed phages in the supernatant was then assayed, using HfrH as the indicator bacteria. It is seen in Table 3 that the results agree with the electron microscopy counts, i.e., 10^{-3} M Zn^{2+} reduces the adsorption of f1 phages to about 20% of the control. In contrast, 10^{-3} M Zn^{2+} exerts no detectable effect on the adsorption of isometric RNA phage MS2 (Table 4). Since the conditions of these experiments were different from those of Tzagoloff and Pratt (11) and Paranchych and Graham (9), it does not seem worthwhile to compare the rates of adsorption with theirs at this point.

TABLE 2. *Effect of 10^{-3} M Zn^{2+} on the number of R17 phages attached to unit lengths of F pili as measured in the electron microscope*[a]

Determination[a]	Control (no added Zn^{2+})	$+10^{-3}$ M Zn^{2}
No. of unit lengths of F pilus on which phage were counted	$N_1 = 44$	$N_2 = 49$
Total no. of R17 phages counted	$\sum X_1 = 413$	$\sum X_2 = 478$
Avg no. of R17 phages attached to a unit length of F pilus	$\bar{X}_1 = 9.4$	$\bar{X}_2 = 9.8$
Sum of squares	$\sum X_1^2 = 4109$	$\sum X_2^2 = 4980$
Sample variance	$s_1^2 = 5.56$	$s_2^2 = 6.88$

[a] Students t test, $t = 0.764$; degrees of freedom, $v = 91$; probability, ≈ 0.45; calculated by the method indicated in reference 10.

TABLE 3. *Inhibition of adsorption of f1 phages by 10^{-3} M Zn^{2+} as measured by plaque assay*

Expt	Host (concn, cells/ml)	Percent of phages absorbed
1		
Control 1	F⁻W1-3 (1.0×10^9)	0
Control 2	HfrH (3.0×10^8)	43.3
$+10^{-3}$ M Zn^{2+}	HfrH (3.0×10^8)	9
2		
Control 1	F⁻W1-3 (1.1×10^9)	0
Control 2	HfrH (2.0×10^8)	26.5
$+10^{-3}$ M Zn^{2+}	HfrH (2.0×10^8)	5.9

From these results one might expect that, when DNA phages are plated in agar containing 10^{-3} M Zn^{2+}, the number of plaques might decrease compared to the control (no added Zn^{2+}), or at least the plaque size might be reduced. As it turned

TABLE 4. *Effect of 10^{-3} M Zn^{2+} on the adsorption of MS2 phages as determined by plaque assay*

Expt	Host (concn, cells/ml)	Percent of MS2 phages adsorbed
1		
Control 1	F⁻W1-3 (3×10^8)	0
Control 2	HfrH (3×10^8)	90
Plus 10^{-3} M Zn^{2+}	HfrH (3×10^8)	87
2		
Control 1	F⁻W1-3 (1.0×10^8)	0
Control 2	HfrH (2.8×10^8)	92
Plus 10^{-3} M Zn^{2+}	HfrH (2.8×10^8)	91.2
3		
Control 1	F⁻W1-3 (4.0×10^8)	0
Control 2	HfrH (1.7×10^8)	87
Plus 10^{-3} M Zn^{2+}	HfrH (1.7×10^8)	86

out, however, this was not the case. We detected no significant difference in plating efficiency of f1 phages when they are mixed with bacteria and are plated with or without 10^{-3} M Zn^{2+} in the medium. The number and sizes of plaques were essentially the same whether 10^{-3} M Zn^{2+} was present or not. This result seems to suggest that there is a constant competition between DNA phages and Zn^{2+} for the binding sites at the tips of F pili, and that eventually the irreversible reaction of the DNA phages prevails over the effect of Zn^{2+}. Once the filamentous phages are adsorbed, they can infect the host and go on to form daughter particles without appreciable restriction by Zn^{2+}. This agrees with the observation (11) that 10^{-3} M Zn^{2+} has no detectable effect on phage production subsequent to adsorption.

Using both the electron microscope and plaque assays, we have shown that, whereas 10^{-3} M Zn^{2+} has no detectable effect on the adsorption of the isometric RNA phages to the sides of F pili, we do confirm the conclusion of Tzagoloff and Pratt (11) that 10^{-3} M Zn^{2+} inhibits the adsorption of filamentous DNA phages to the tips of F pili. Our experiments do not show whether the Zn^{2+} acts on the tips of F pili or on the A protein which is located on one tip of the filamentous phage (6) and which is responsible for its adsorption. However, since we have shown (8) that 10^{-3} M Zn^{2+} acts reversibly on the tips of F pili to inhibit their interaction with receptor spots on female bacteria, one might suppose that Zn^{2+} renders pili tips unsuitable for the adsorption of the phage A protein as well.

This investigation was supported by grant GB-29291X from the National Science Foundation; by Public Health Service grants CA-06927 and RR-05539 from the National Cancer Institute and the Division of Research Facilities and Resources, respectively; and by an appropriation from the Commonwealth of Pennsylvania to The Institute for Cancer Research.

LITERATURE CITED

1. Brinton, C. C. 1965. The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram-negative bacteria. Trans. N.Y. Acad. Sci. Ser. III. 27:1003–1054.
2. Caro, L. G., and M. Schnös. 1966. The attachment of the male-specific bacteriophage f1 to sensitive strains of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 56:126–132.
3. Crawford, E. M., and R. F. Gesteland. 1964. The adsorption of bacteriophage R17. Virology 22:165–167.
4. Ippen, K. A., and R. C. Valentine. 1967. The sex hair of *E. coli* as sensory fiber, conjugation tube, or mating arm? Biochem. Biophys. Res. Commun. 27:674–680.
5. Knolle, P. 1967. Evidence for the identity of the mating-specific site of male cells of *Escherichia coli* with the receptor site of an RNA phage. Biochem. Biophys. Res. Commun. 27:81–87.
6. Marvin, D. A., and B. Hohn. 1969. Filamentous bacterial viruses. Bacteriol. Rev. 33:172–209.
7. Novotny, C., W. S. Knight, and C. C. Brinton. 1968. Inhibition of bacterial conjugation by ribonucleic acid and deoxyribonucleic acid male-specific bacteriophages. J. Bacteriol. 95:314–326.
8. Ou, J. T., and T. F. Anderson. 1972. Effect of Zn^{2+} on bacterial conjugation: inhibition of mating pair formation. J. Bacteriol. 111:177–185.
9. Paranchych, W., and A. F. Graham. 1962. Isolation and properties of an RNA-containing bacteriophage. J. Cell. Comp. Physiol. 60:199–208.
10. Simpson, G. G., A. Roe, and R. C. Lewontin. 1960. Quantitative zoology. Harcourt, Brace and Company, New York.
11. Tzagoloff, H., and D. Pratt. 1964. The initial steps in infection with coliphages M13. Virology 24:372–380.