ADSORPTION OF BACTERIOPHAGES SPECIFIC FOR PSEUDOMONAS AERUGINOSA R FACTORS RP1 AND R1822

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SUMMARY

Short, thick pili were found by electron microscopy on bacteria carrying the P group drug resistance plasmids RP1 and R1822. The R1822-specific phage PRR1 was seen to adsorb to the bases of the pili. Three RP1-specific phages, one filamentous (Pf3), and two with very thick capsids (PR3, PR4), were seen to attach all around the surface of P. aeruginosa cells, and were thought to be somatic, since pilus phages appear to be strictly polar on this species. PR3 and PR4 also lysed a strain of E. coli containing an N group plasmid, suggesting a relationship between the N and P group plasmids.

INTRODUCTION

Following the recent isolation of an RNA phage (PRR1) specific for the Pseudomonas aeruginosa P group R factor R1822 (12), three morphologically different phages have been obtained for the related plasmid RP1 by V. Stanisich (Monash University, Australia, personal communication). While their structure is not primarily the subject of this communication, a brief description is necessary. Pf3 is a typical, unusually short (760 nm) filamentous type. Isolates PR3 and PR4 are similar to one another, being hexagonal in outline and having a 65 nm diameter capsid with a thickened inner layer (Fig. 8) like the lipid-containing phage PM2 (8). Unlike PM2, PR3 and PR4 are thought to have a short (60 nm) easily detached tail, not usually visible; this remains to be confirmed. Preliminary

electron microscope observations on the adsorption of all four phages to sensitive bacteria are described here.

MATERIALS AND METHODS

Bacteria and phages were very kindly supplied as follows: E. coli CR34 R1822 and phage PRR1 by Dr R. H. Olsen (12); phages Pf3, PR3 and PR4 by Dr V. Stanisich; E. coli AS19 and AS19 RP1 (15), and P. aeruginosa 18S and 18S RP1 by Mr T. L. Pitt, Central Public Health Laboratory, London; E. coli J5-3 and J5-3 R199 by Mr R. Thompson (Edinburgh). P. aeruginosa K/1PO4⁻RP1 is a pilus-less mutant of strain K (5).

The spot test was used for determining bacteriophage lytic activity. Antiserum against RP1 pili was prepared from P. aeruginosa 18S RP1: a cell free extract from double agar layer plates of bacteria was used for inoculating rabbits. Bacterial cells with adsorbed phages were prepared for electron microscopy by mixing log. phase bacteria with phage to a multiplicity of between 5 and 20. After 20 minutes at 37°C, cells were mounted on support grids (5) and negatively stained.

RESULTS AND DISCUSSION

The lytic activities of the four phages are compared in Table 1, showing their RP1- and R1822-specificity on strains of both P. aeruginosa and E. coli. PR3 and PR4 also have some limited activity on E. coli carrying the R factor R199 (11), an N compatibility group plasmid. Confirmation was obtained by plating dilutions of PR4 on E. coli J5-3 R199. Very turbid plaques were obtained; they contained virions (as Fig. 8) when extracted for electron microscopy (1). This result is of particular interest because the DNA of RP1 (P group) has a 50%

TABLE 1

LYTIC ACTIVITY OF BACTERIOPHAGES AS SHOWN BY THE SPOT TEST

Strain	Sensitivity to phages*			
	PR3	PR4	Pf3	PRR1
P. aeruginosa				
18S	-	-	-	-
18S RP1	+	+	+	+
K/1PO4⁻RP1	+	+	+	+
E. coli				
AS19	-	-	-	-
AS19 RP1	+	+	+	+
CR34 R1822	+	+	+	+
J5-3	-	-	-	-
J5-3 R199	(+)	(+)	-	-

* + indicates clearing and sensitivity to a phage, (+) indicates partial clearing and sensitivity, and - no clearing and resistance. Phages were grown on strains not carrying bacteriocins active on the test bacteria.

homology with DNA of the N group plasmid 390 (10).

R-specific pili were identified by labeling with antibodies: one part of antiserum (see above) was incubated with five parts of log. phase bacterial culture for two hours. After mounting for electron microscopy, R-specific pili were sought on E. coli strains. Fig. 1 shows a heavily labeled pilus

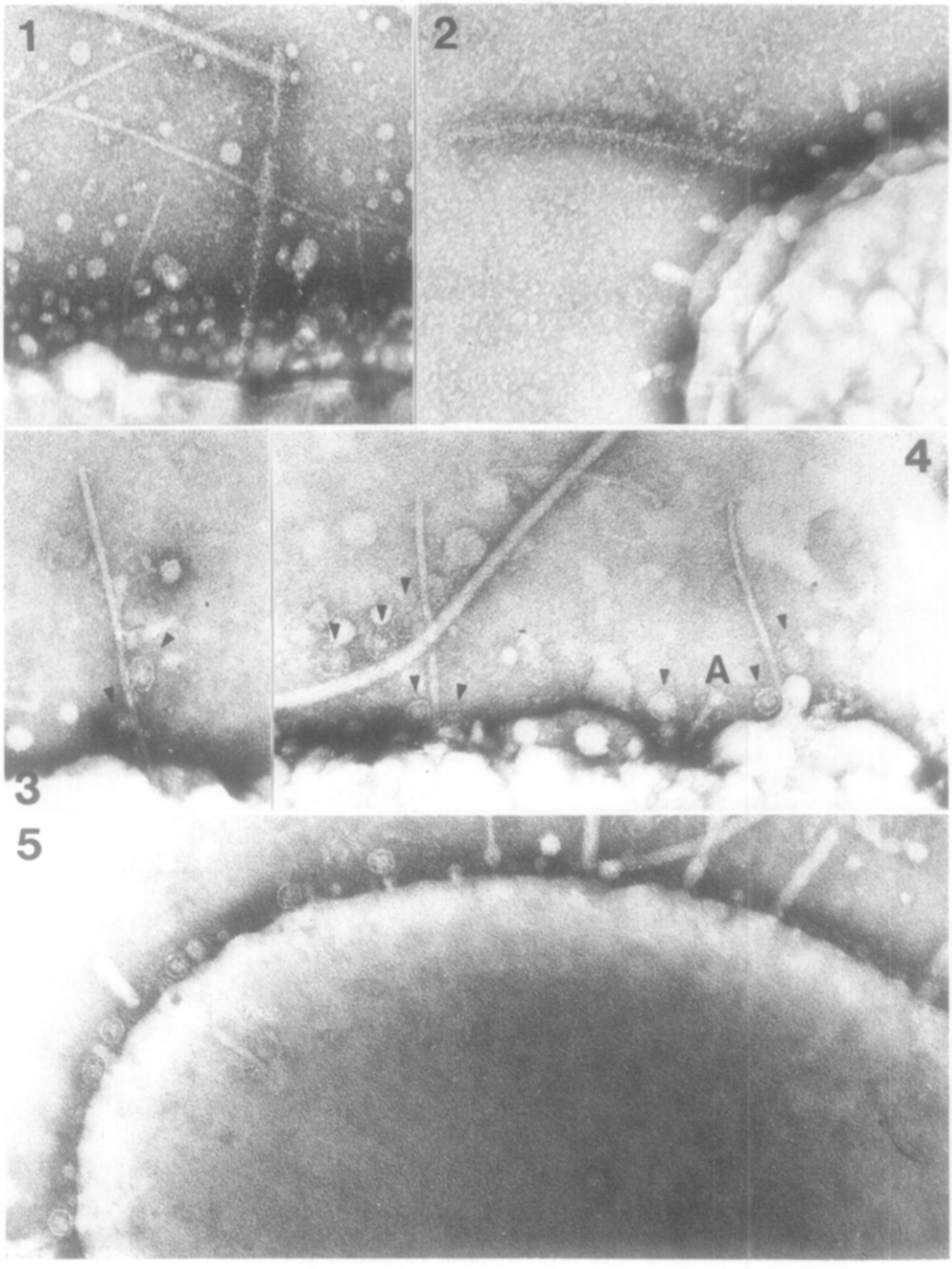

Fig. 1. Antibody-labeled RP1 pilus on E. coli AS19 RP1 with uncoated "common" pili and a flagellum (top). X 125,000. Fig. 2. E. coli CR34 R1822 prepared as for Fig. 1. X 125,000. Figs. 3, 4. E. coli CR34 R1822 with PRR1 virions (arrowed) attached to R1822 pili, the cell surface, and flagella. "A" is a bacteriocin rod. X 125,000. Fig. 5. P. aeruginosa 18S RP1 with PRR1 adsorbed strictly to the pole. X 125,000.

on an AS19 RP1 cell; it is clearly distinguishable from the unlabeled "common" pili with which this strain is covered. With strain CR34 R1822 (no common pili), R pili (Fig. 2) were more obvious, and seemed to appear all around the cell as opposed to the apparent polar location of those on P. aeruginosa. The labeling of R1822 pili with anti-RP1 indicates an expected relationship, since RP1 and R1822 may be the same plasmid (9).

The RNA phage PRR1 generally adsorbed to E. coli CR34 R1822 at the bases of the R pili (Figs. 3, 4). This is similar to the RNA phage PP7 (2), which was found to adsorb to the bases of pili (not associated with any known plasmid) on P. aeruginosa PAO1. It was shown that the pili had retracted, pulling the virions to the cell surface (3,4). A very few PRR1 virions were found at the cell surface away from the pili, an example being shown in Fig. 4; some particles are also attached (presumably non-specifically) to a flagellum. The cell in Fig. 3 was typical, and had no virions at the cell wall away from the single pilus. It is considered that these micrographs show that PRR1 adsorbs to pili as do all other known RNA phages. The unusually short R pili are about 9 nm thick (9.5 nm for F-pili), which is thicker than the 6 nm of the "normal" polar pili usually associated with P. aeruginosa (4).

With P. aeruginosa 18S RP1, PRR1 virions attached preferentially to the polar surface of the cell (Fig. 5) as do a number of P. aeruginosa tailed pilus phages (6, 7, 14). "Normal" P. aeruginosa pili, like RP1 pili, are located at the pole, and it is thought that they pull the phages to the cell surface as they retract and disappear into the cell. Alternatively the

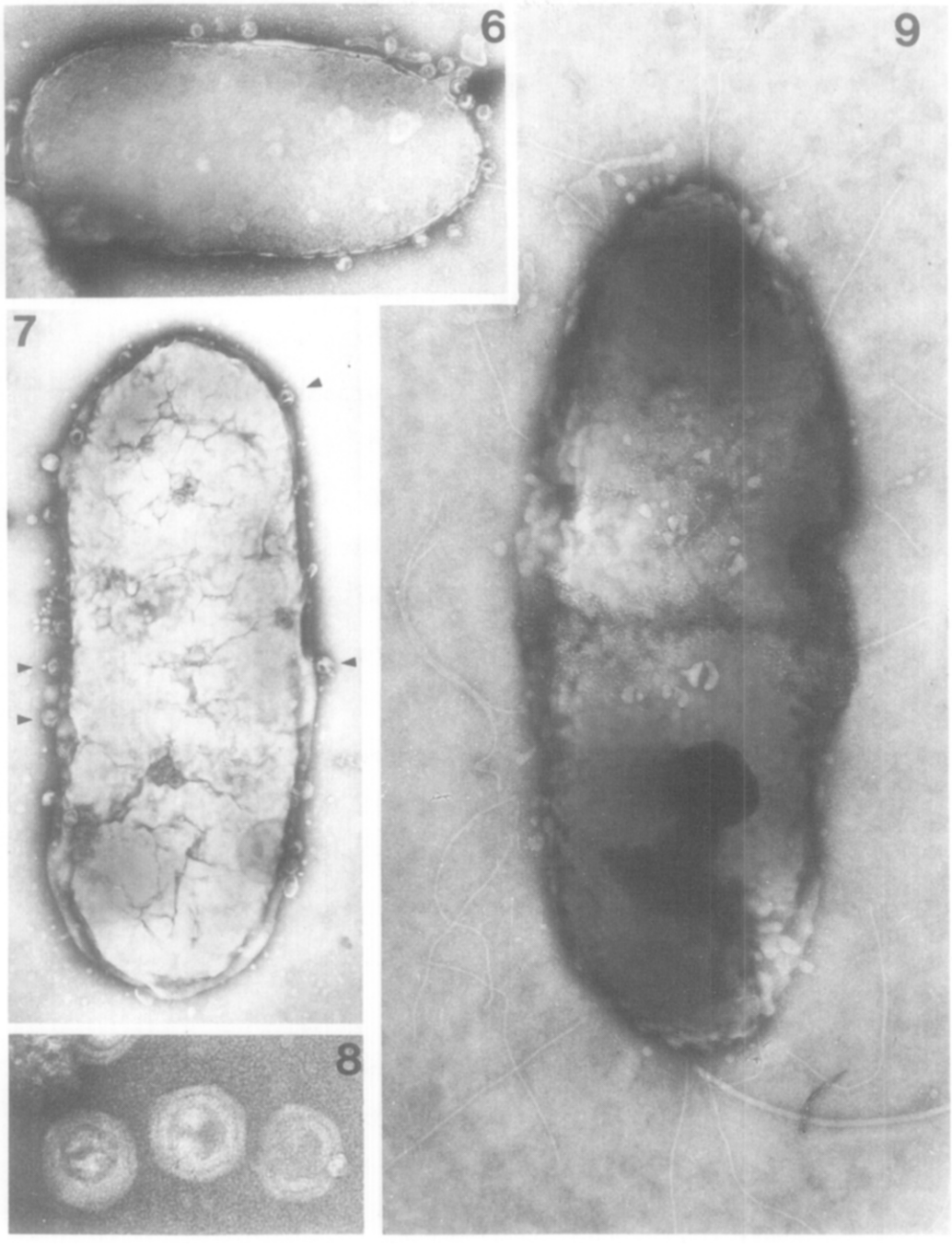

Fig. 6. Phage PR4 adsorbed to P. aeruginosa K/1P04⁻RP1. X 36,000. Fig. 7. Phage PR3 adsorbed to E. coli CR34 RP1. Some of the virions are arrowed. X 36,000. Fig. 8. PR4 virions extracted from a spot test "plaque" on E. coli J5-3 R199. Suspected tails may have become detached. X 200,000. Fig. 9. P. aeruginosa K/1P04⁻RP1 with adsorbed filamentous phage Pf3. X 50,000.

pili could break off. This situation is different to E. coli R pili with adsorbed PRR1, where retraction or breakage does not seem to occur. PRR1 is a typical RNA phage about 26 nm in diameter, which is near the estimate of Olsen and Thomas (13).

Phage PR3 virions adsorbed all around 18S RP1 cells, as was the case with the similar isolate PR4 adsorbed to strain K/1P04⁻RP1; the virions frequently had a slight preference for the pole (Fig. 6). If this phage adsorbed to RP1 pili, one would expect a strictly polar location since this is where the pili are located. Phage PR3 attached to the cell wall of E. coli CR34 R1822 with no preference for the pole, and in a manner typical of somatic phages independently of any visible pili (Fig. 7). Similar results were obtained when the filamentous phage Pf3 was adsorbed to P. aeruginosa strain K/1P04⁻RP1 (Fig. 9). Pf3 thus appears to be somatic like the N group R factor filamentous phage Ike (Iyer, personal communication).

The implication of these results is that RP1 and R1822 can code for two different phage receptors: pili for PRR1 and somatic receptors for Pf3, PR3 and PR4. Alternatively it could be that Pf3, PR3 and PR4 adsorb to pili which retract fully or disappear by breaking off, or perhaps they adsorb to very short pili which are not visible. This explanation would be more in agreement with the results of Stanisich (personal communication), which show that mutants selected as resistant to any one of the four phages are always resistant to the rest, suggesting a common receptor. However, the different locations on P. aeruginosa of R pili (polar) and adsorbed Pf3, PR3 and PR4 virions (peripheral) suggest two different kinds of receptor, the synthesis of which may be under the same control.

REFERENCES

1. Bradley, D. E. J. Roy. Microscop. Soc. 84, 257 (1965).
2. Bradley, D. E. J. Gen. Microbiol. 45, 83 (1966).
3. Bradley, D. E. Biochem. Biophys. Res. Commun. 47, 142 (1972).
4. Bradley, D. E. J. Gen. Microbiol. 72, 303 (1972).
5. Bradley, D. E. Can. J. Microbiol. 19, 623 (1973).
6. Bradley, D. E. J. Virol. 12, 1139 (1973).
7. Bradley, D. E. and Pitt, T. L. J. Gen. Virol. In press.
8. Espejo, R. T. and Canelo, E. S. Virology 34, 738 (1968).
9. Holloway, B. W. and Richmond, M. H. Genet. Res. Camb. 21, 103 (1973).
10. Ingram, L. C. J. Bacteriol. 115, 1130 (1973).
11. Meynell, E. and Datta, N. Genet. Res. Camb. 7,134 (1966).
12. Olsen, R. H. and Shipley, P. J. Bacteriol. 113, 772 (1973).
13. Olsen, R. H. and Thomas, D. D. J. Virol. 12, 1560 (1973).
14. Pemberton, J. M. Virology 55, 558 (1973).
15. Sekiguchi, M. and Iida, S. Proc. Nat. Acad. Sci. U.S.A. 58, 2315 (1967).