EVIDENCE FOR THE RETRACTION OF PSEUDOMONAS AERUGINOSA RNA PHAGE PILI

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SUMMARY

The pilus retraction model suggests that RNA phage pili retract when virions adsorb to their sides. The particle nearest the base is pulled against the cell surface and stops further retraction. If one phage per pilus were adsorbed, a length reduction averaging 50% over a number of pili would be predicted due to adsorption at random points along them. Electron microscopy is used here to measure the lengths of polar pili of P. aeruginosa before and after RNA phage adsorption. 40-50% reductions are found and length distribution curves indicate that retraction has occurred.

INTRODUCTION

It is uncertain how Escherichia coli F-pili and the polar pili (RNA phage receptors) of Pseudomonas aeruginosa function. Two systems are currently favoured, the retraction (5, 7) and the conduction models (4). The first suggests that contact with an RNA or filamentous bacteriophage or recipient cell causes the pilus to retract, the structural units being dissociated by some form of depolymerization mechanism at the base. The genetic transfer of F is achieved by the mating cells being drawn into contact allowing the formation of a classical conjugation bridge (1). Filamentous phages adsorbing to the pilus tip are supposedly drawn right into the cell, and RNA phages are pulled into contact with the cell surface

where RNA penetration occurs. With the second model, F transfer takes place down the pilus which acts as a simple tube for nucleic acid conduction. Filamentous phage DNA passes right down the tube from the tip, and RNA phage RNA enters the pilus lumen through the side and is transferred to the cell. Much evidence is available to favour both concepts. In this communication, a direct approach is used in an attempt to demonstrate retraction. The pili should shorten by retraction after RNA phage adsorption at a low multiplicity of infection. Length measurements are therefore made before and after adsorption to determine whether or not there is a reduction in the mean value. The model is illustrated in Fig. 1.

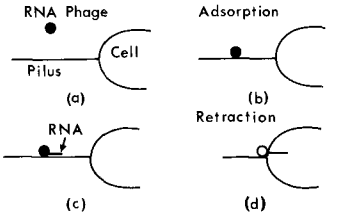

Fig. 1. Hypothetical scheme for the adsorption of a single RNA phage virion to a pilus receptor which subsequently retracts. (a) Random approach of RNA phage. (b) Virion adsorbs at any point along the pilus; this would average out to the midpoint over a number of pili. (c) A short length of viral RNA is ejected onto the pilus surface where it is RNAse-sensitive. (d) At the same time retraction is initiated, pulling the phage against the cell and the RNA through the cell wall; the average protruding length of a number of pili after adsorption would thus be half the value obtained before adsorption.

P. aeruginosa is used because indications of pilus retraction have been encountered before (2). In addition, the pili are more numerous than E. coli F-pili, and their polar position simplifies both length measurements and the interpretation of

results; no correction need be applied for the obscuring effects of the cell body as with E. coli (8).

MATERIALS AND METHODS

P. aeruginosa strains PA038 and PA01264 (6) were kindly supplied by Professor B. W. Holloway. Strain PA038 is RNA phage-sensitive, but strain PA01264 is not, though the virions adsorb to its pili (2), and the pili of both strains are serologically related (Bradley, unpublished). For these reasons strain PA01264 was thought to be useful as a control whose pili might not retract. The RNA phage used was PP7 (3). Standard methods were employed for culturing the bacteria and preparing phage suspensions. A 5 hr shake culture of bacteria in Oxoid Nutrient Broth was diluted to 2 X 10^8 organisms/ml with broth and mixed with an equal volume of PP7 at 1 X 10^{12} p.f.u./ml. This high m.o.i. gave rapid adsorption, but, because of the high pH of the culture (8.4), only virions which were presumably irreversibly adsorbed remained attached to the pili to give the desired level of 1-2 phages per pilus. After 10 min shaking at 37°C, carbon-coated grids were floated on the mixture for 5 min. Washing and negative staining with sodium phosphotungstate (NaPT), or shadowing followed. Unlabelled samples of bacteria were prepared simultaneously. About 50 random poles were photographed in the electron microscope and pilus lengths were measured on calibrated enlargements to an accuracy of about 50 nm. Counts of pili/cell were also carried out.

TABLE I

DEGREE OF PILIATION AND AVERAGE LENGTHS OF PILI FOR PHAGE-LABELLED AND UNLABELLED PSEUDOMONAS AERUGINOSA

Strain	Treatment	% Cells piliated	Average pili/cell	Mean length (nm)	Reduction* in length (%)
PA038	Unlabelled, NaPT.	3.8	0.1	1117	0
PA038	Phage-labelled, NaPT.	100	7.0	662	40.5
PA038	Unlabelled, shadowed.	6.0	0.06	1084	0
PA038	Phage-labelled, shadowed.	94	5.6	545	50
PA01264	Unlabelled, NaPT.	98	6.6	1064	0
PA01264	Phage-labelled, shadowed.	96	9.0	1061	0

*The percentage length reduction is with reference to the unlabelled sample in each pair of observations.

RESULTS AND DISCUSSION

Table 1 shows the relationship between piliation (% of cells with pili and the average number of pili/cell) and the average length of pili. Each pair of observations shows the comparative results before and after phage-labelling. It can be seen that unlabelled PA038 cells, both negatively stained and shadowed, have far fewer pili than PP7-labelled ones. From the mean lengths given, it can be seen that a substantial length reduction after labelling coincides with an increase in

piliation in the case of PA038, but not with PA01264. This result is interpreted as indicating that the preparation process (perhaps a drying effect) causes the unlabelled pili to retract completely into the cell, thus disappearing from sight. The few visible unlabelled pili are considered to be defective in some way. Complete retraction of the phage-labelled ones is, however, prevented by adsorbed virions. It is also considered that the pili of strain PA01264 have lost their ability to retract.

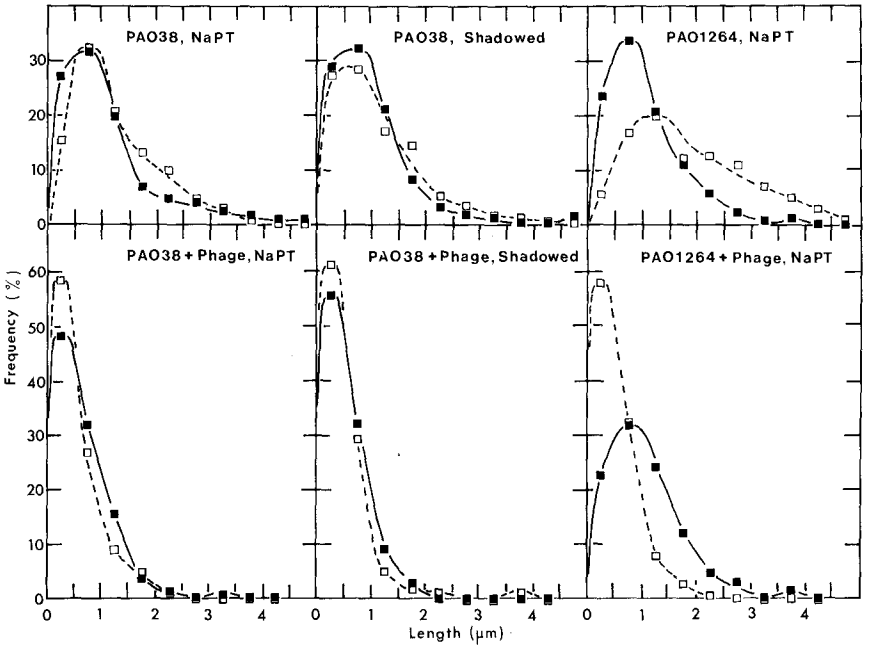

Fig. 2. Frequency distribution curves of pilus length measurements before and after PP7-labelling. ■———■ Experimental curves obtained from preparations as described in each graph. □- - - -□ Theoretical curves obtained by halving each unlabelled measurement (plotted on lower graphs) or doubling each labelled measurement (plotted on top graphs). Theoretical curves demonstrate what should happen if the predictions illustrated in Fig. 1 are correct. The curves for the PP7-resistant control strain PA01264 should not match.

To test these hypotheses further, length distribution curves of the same samples were plotted (Fig. 2). Experimental results are shown as continuous lines, and broken lines represent theoretical distributions obtained as follows. Assuming that phages adsorb at random along the pili (Fig. 1), and that a single virion can block pilus retraction at the point of adsorption, a mean length reduction of 50% would be expected after the adsorption of one phage/pilus. Theoretical length distributions representing this 50% retraction can be obtained by halving each measurement of the unlabelled pili. Conversely from PP7-labelled samples, one can construct theoretical curves for the unretracted length by doubling each measurement. If the two sets of curves match, then a 50% length reduction will have occurred.

It is considered that the only alternative cause for this type of length reduction other than retraction is pilus breakage, perhaps at the point of adsorption (9). However, this is at variance with the piliation data in Table 1, since if breakage occurred, there would have to be the same number or fewer pili after phage-labelling, not more. Also virions were clearly visible at the PA038 pilus bases (Fig. 3) as predicted in Fig. 1, and it is unlikely that the serologically similar PA01264 pili are resistant to breakage. Paranchych *et al* (9) state that pilus retraction after phage adsorption should show up in length distribution curves as a downshift in modal length. This is clearly the case in Fig. 2 with PA038 only (maxima: unlabelled, 750 nm; PP7-labelled, 250 nm).

It is considered that these results provide direct

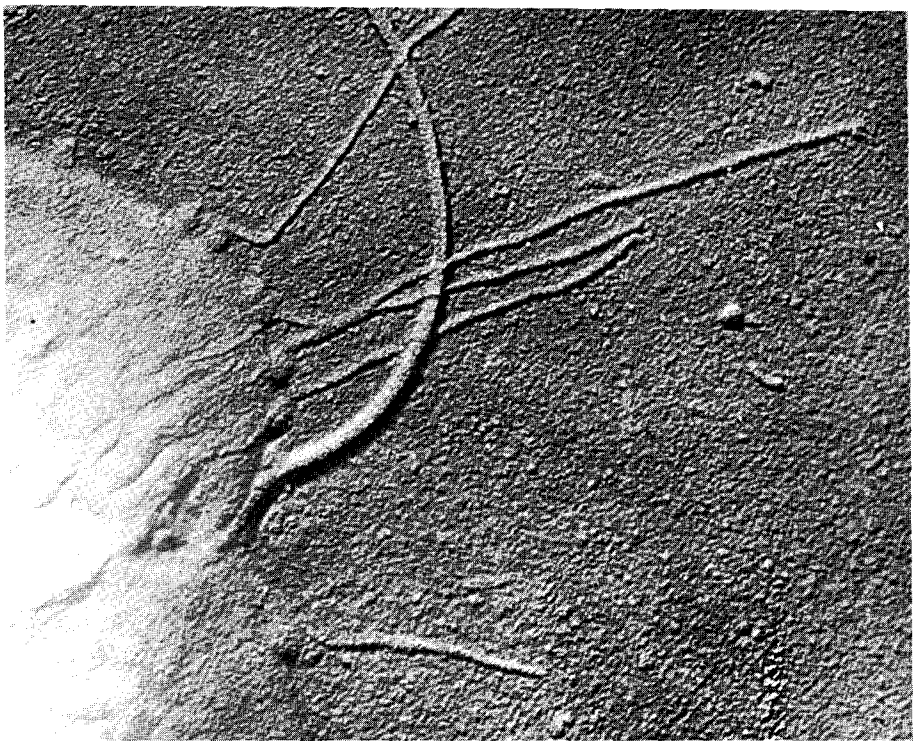

Fig. 3. Shadowed preparation of strain PA038 after PP7-labelling at about one phage/pilus. Virions are at the bases of the pili, not randomly distributed along them as was the case with strain PA01264. X 93,500.

evidence of pilus retraction, which is believed to be very rapid. The results do not show whether it is initiated by phage adsorption or is due entirely to drying or contact with the support film. However, it would certainly seem that retraction is a necessary step in RNA penetration since phage resistance in PA01264 is linked with a lack of shortening of pili after adsorption. These results only apply to P. aeruginosa, and preliminary counts of F-pili show only a doubling rather than a 70-fold increase in piliation after RNA phage labelling.

Perhaps the much thicker F-pili are dissociated more slowly or are insensitive to drying effects.

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