GENERAL PROPERTIES OF F-PILI*

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The first electron micrographs of F-pili (Crawford and Gesteland, 1964) showed that these thin, hair-like appendages grew out from the surface of the male cell, sometimes reaching several microns in overall length. Brinton, Gemski, and Carnahan (1964) observed that each male cell produced only a few intact F-pili; they also observed free F-pili fragments of various lengths which appeared to have been broken from the cell during growth. These broken pieces of pili still adsorbed phage particles. In certain cases we have observed (unpublished data) that pieces of pili as short as 1000 Å adsorbed phages. Higher resolution micrographs showed what appeared to be a hollow core running the length of F-pili (Valentine and Strand, 1965). These data suggested that F-pili were narrow, hollow tubes, with no precise "unit length" requirement for biological activity (phage adsorption). That is, F-pili appeared to be made of a number of repeating structural units, each of which was capable of binding phage. The data presented below confirm this general conclusion and show: (1) F-pili are rather low density structures, banding at a buoyant density of approximately 1.19 in CsCl; (2) F-pili are rapidly destroyed by lipid solvents and heat; and (3) F-pili apparently vary considerably in length, the longer segments being readily broken by shearing into smaller active fragments still capable of adsorbing phage.

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Results and Experimental

F-pili Preparation. F-pili were obtained from the supernatants of male cultures of E. coli strain B/r (Brinton, et al., 1964) grown with shaking in broth for 6-8 hours. By this time the free F-pili concentration was maximum in the culture (Ippen and Valentine, 1965). Thus, the F-pili used in the experiments described below are those which have been broken from the cells during growth or during sedimentation. These F-pili were not purified further. The use of such pili preparations made it less likely that any component associated with the F-pili would be damaged or removed.

It should also be noted that male B/r synthesizes only F-pili, but no common pili (Brinton et al., 1964). F-pili were assayed by the filtration procedure described by Ippen and Valentine (1965). The exact conditions of the assay varied depending on the particular experiment and the specific activity of the phage.

Buoyant Density Measurements. F-pili from E. coli B/r supernatants were banded by adding 1.2 g of CsCl to 4 ml of supernate and centrifuging in a Spinco model L ultracentrifuge at 100,000 x g for 40 hours. The fractions were collected and assayed for F-pili using the filtration procedure. Assay samples small enough to avoid interference by CsCl were used.

Fig. 1 shows that the F-pili banded with a peak corresponding to a mean density of 1.197 as calculated from the refractive index. The CsCl solution contained culture broth which may have caused some shift of density; however, other measurements made with partially purified pili in water gave closely similar values.

Sensitivity to Lipid Solvents and Heat. F-pili in cell-free supernatants have been found to be extremely sensitive to lipid solvents such as chloroform, benzene and CCl_4. The effect on F-pili

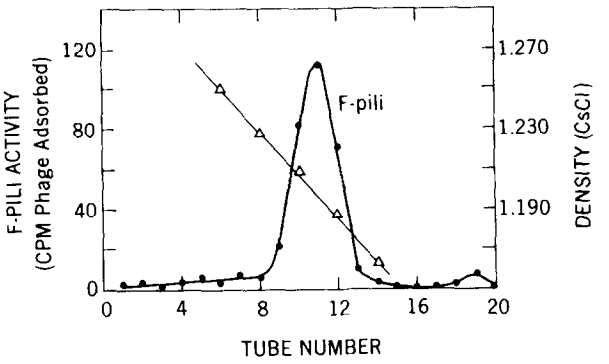

Fig. 1. The buoyant density of F-pili in CsCl. Details in text.

activity (phage adsorption) of treatment of the sample with chloroform is shown in Fig. 2. For this experiment several drops of chloroform were added to 10 cc of F-pili preparation in order to saturate the solution with chloroform. Samples were removed and assayed. F-pili appeared to be rapidly destroyed by this procedure.

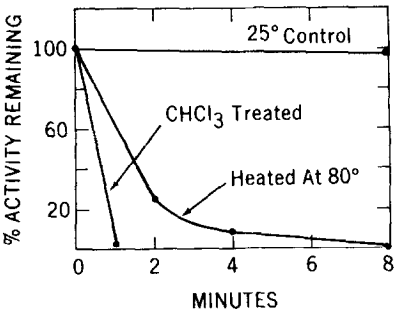

Fig. 2. Sensitivity of F-pili to chloroform and heat. See text for details. Samples assayed for phage adsorption by the filtration method. Note the rapid drop in activity after chloroform treatment.

In a control experiment to rule out the possibility that traces of chloroform were affecting the assay or filter, a concentrated F-pili preparation was treated with chloroform as above and then diluted 1:100 before assay. Similar results were obtained. In other experiments it was observed that F-pili-phage complexes pre-formed by incubation of

phage and F-pili were disrupted by chloroform treatment.

Heating F-pili to 80°C also destroyed their ability to adsorb phage. As shown in Fig. 2, only 10 per cent of the phage adsorbing activity remained after heating at 80° C for 4 minutes.

Sucrose Gradient Profiles. The sedimentation pattern of F-pili in a sucrose gradient (Fig 3) reveals extreme heterogeneity in their molecular weight. The majority of pili sediment in a peak moving at a rate somewhat faster than that of the rod-shaped phage f1, but phage absorbing activity is spread throughout the gradient. Some F-pili appear to have been pelleted by this procedure.

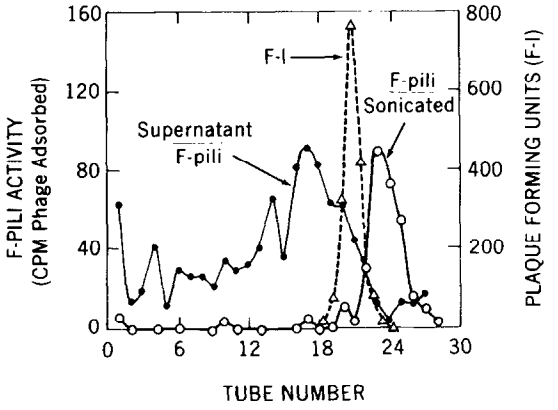

Fig. 3. Sucrose gradient profiles of F-pili. Sedimentation of F-pili was carried out by layering 2 ml of F-pili on top of a sucrose gradient (20-40%) and centrifuging for 9 hr. at 64,000 x g using the SW-25 swinging bucket rotor. The three samples were sedimented in separate tubes. The phage f1 was used as a marker because of its similar morphology. It has been calculated to have a molecular weight of approximately 11 x 10^6 (Zinder et al., 1963). The sonicated sample was prepared by treating a cell-free F-pili preparation for 5 min. in a 10 kc sonicator. Note the marked shift in sedimentation after sonication.

The different peaks may represent pili of various lengths, since sonication of the supernatant eliminates them and produces a new, narrower peak, sedimenting slightly slower than phage f1 (Fig. 3). The different lengths observed before sonication would then be due to spontaneous breakage during growth and sedimentation of the cells.

The total activity of the supernatant is reduced by almost one-half during sonication. This may be caused by denaturation of pili due to local heating, or sonication may reduce pili to fragments which are small enough to be passed by the filter used in the assay procedure. Pili of this length could be present near the top of the gradient but would not be detected.

Discussion

The properties of F-pili described above clearly bear on the questions of their similarity to type I pili (Brinton, 1959) and possible DNA content. First of all, their sedimentation pattern in sucrose and its response to sonication are consistent with the morphology of these structures as seen in electron micrographs, where they appear as thin rods of varying length. Whether the fragments present in the sonicated peak represent a fundamental unit of pilus structure is uncertain. Electron micrographs of the sonicated material may help to settle this point.

F-pili seem different from type I pili. Both the sensitivity to lipid solvents and the low buoyant density suggest that F-pili contain lipid, whereas type I pili have been stated to be composed solely of protein (Brinton et al., 1964). The heat sensitivity of F-pili may further distinguish them from type I pili which retain their characteristic appearance even after heating at 95° (Brinton, 1959). It is possible, but does not seem likely, that the same may be true of F-pili; that is, that heating destroys phage adsorption capacity while leaving their over-all morphology unchanged.

Finally, it appears improbable that free F-pili contain, or are associated with a large DNA component. The DNA containing coliphage f1, for example, while composed of only about 10 per cent DNA has a

density under these conditions of 1.29, compared to a density of 1.197 for F-pili. It can be argued, however, that a structure sufficiently rich in lipid might show a low buoyant density even though it contained some DNA. A definite answer to this question will require chemical characterization of F-pili.

References

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