THE FATE OF THE PROTEIN COMPONENT OF BACTERIOPHAGE fd DURING INFECTION

E. Trenkner, F. Bonhoeffer and A. Gierer
Max-Planck-Institut für Virusforschung, Tübingen

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1. INTRODUCTION

In this paper it will be shown that the small fibrous phage fd invades the host cell with its protein. Two lines of evidence will be presented: the ratio of protein to DNA in infected bacteria is found to be the same as that in the intact phage. This ratio remains unaffected by various kinds of treatment intended to remove external phage, external phage protein, and F-pili. The total amount of both protein and DNA is equivalent to about one infecting phage per infective center. In the second type of experiment, bacteria were infected with a preparation of phage in which thymine is replaced by 5'-bromo-uracil (BU) in the DNA, whereas the protein is labelled with radioactive amino acids. Density gradients show that radio-activity originally connected with the heavy infecting phage appears, after infection, in the light phage progeny. Therefore, either phage protein or its amino acids are utilized for the synthesis of progeny phage. This mechanism implies that viral protein enters the cells with the infecting phage.

2. MATERIALS AND METHODS

Strains: The rod shaped bacteriophage fd was used in all the experiments. It was grown and purified according to the methods of Hoffmann-Berling and Marvin (1963). Hfr U 165/1 a thymine

deficient derivative of the E. coli strain Hfr H was used for the production of radioactively labelled phages. E. coli K12 Hfr C3300 was used as the host in all experiments and as the indicator strain in plaque tests.

Media: TPG-medium similar to that described by Sinsheimer et al. (1962); T-medium (Hoffmann-Berling and Marvin, 1963); AP-Buffer (Tzagoloff and Pratt, 1964), and a slightly modified form of adsorption medium (AM) described by Hershey and Chase (1952).

Nagarse: Manufactured in Japan Teikoku Chemical Industry Co.

Preparation of doubly-labelled and ^{32}P-labelled radioactive Phages

Hfr U 165/1 cells and fd-phage were incubated together in 60 ml modified TPG-medium containing 5γ thymine/ml at 37°C under mild aeration until the cell concentration reached 3×10^8 cells/ml. Then the cells were collected by centrifugation, washed and resuspended in 60 ml TPG-medium containing 5γ thymine/ml 0.3 mC ^3H thymine (15,000 mc/mM) and 3 mC ^{35}S. The cells were incubated for 6 hr. at 37°C, and then centrifuged. The supernatant containing the phages was concentrated 4-fold with a rotatory evaporator run at 30°C, and then incubated at 37°C for 30 min with 40γ/ml RNase, 40γ/ml DNase and 40γ/ml trypsin. Thereafter, the phages were first purified on a sephadex G-100 column, and then further purified and concentrated in a CsCl gradient (1.2 g CsCl/3 ml). ^{32}P-labelled phages were similarly prepared.

fd Phages with heavy DNA and radioactive Protein

Hfr U 165/1 cells were grown to a concentration of $3 - 5 \times 10^8$ cells/ml in 30 ml TPG containing 10γ thymine/ml and then infected with fd phages at a multiplicity of ten for 60 minutes. Thereafter, the free phages were removed by washing with BU-TPG (TPG containing 40γ BU/ml). The infected bacteria were starved for thymine for 5 min. in 30 ml TPG without thymine and BU at 37°C under mild aeration, and then further incubated in 30 ml BU-TPG at 37°C. In order to obtain pure heavy phages the phages made in the next 40 min. were again removed by washing, and the bacteria were reincubated at a concentration of 6×10^8 cells/ml for an additional 90 min. in 30 ml BU-TPG containing 0.001 mC ^{14}C amino acid mix (40 mC/mM, manufactured by New England Nuclear Corporation). The resulting phages were purified and characterized as described above.

Preparation of the Phage-Bacteria Complex (PhB)

Hfr C3300 cells were grown in T-medium to 3×10^8 cells/ml. Then they were centrifuged, resuspended in an equal volume of T-medium containing 30γ chloramphenicol (CM)/ml and incubated 10 min. at 37°C under mild aeration. Thereafter the cells were infected with doubly-labelled radioactive phages for 30 min. They were washed by centrifugation at 4°C and resuspended in the initial volume of AP-Buffer containing 30γ CM/ml. The PhB formed in this way was subjected to disaggregation methods.

Determination of Radioactivity

The amount of radioactivity was determined in dioxane scintillation fluid. The values were corrected for quenching effects.

Infective Centers

The number of infective centers was determined by diluting infected bacteria in ice-cooled AM containing a 5×10^{-4} concentration of fd specific antiserum, allowing the samples to stand 10 min., and then plating them.

Particle Counts by Electron Microscopy

The concentration of the physical particles was kindly determined by Dr. H. Frank. The method used was a slight modification (unpublished) of the method described by Kellenberger and Arber (1957).

3. EXPERIMENTAL RESULTS

If bacteria are infected with radioactive phage, followed by removal of residual external phage by repeated centrifugation, the ratio of protein to nucleic acid remaining with the infected bacteria was found to correspond to the ratio of protein to DNA of the infecting phage. Incubation with 0.02 M EDTA/ml for 30 min. at 37°C or 5 mg Nagarse/ml for 30 min. at 37°C or blendor treatment for 1 min. at 0°C left this ratio unaffected (Table 1). These treatments remove external residual virus, external viral protein and pili, respectively. The total amounts of phage nucleic acid and protein per infective center have been

Table 1 Phage DNA and Protein per Infective Center
(All data refer to the same experiment)

Column	1	2	3	4	5
		Phage bacteria complex			
	infecting phage	untreated	EDTA treated	Nagarse treated	Blendor treated
DNA ^{3}H cpm/ml	138	140	65	118	131
Protein ^{35}S cpm/ml	78	80	37.6	65	70
Infective Centers/ ml	1.8×10^{7}	2.75×10^{7}	1.5×10^{7}	2.76×10^{7}	2.8×10^{7}
Bacteria/ml	-	3.1×10^{8}	1.5×10^{8}	2.3×10^{8}	2.4×10^{8}
^{35}S Protein per ^{3}H DNA	0.55	0.57	0.58	0.55	0.53
Phage DNA^{+} per Infective Center		1.2	1.0	1.0	1.1
Phage Protein^{+} per Inf.Center		1.2	1.04	1.0	1.05

$^{+}$measured in terms of the amount per phage particle.

calculated from the data given in the first part of Table 1 in conjunction with the plating efficiency of 0.57 of fd as measured by plaque counts and electron microscopy. These amounts are shown in the second part of Table 1 and correspond to the DNA and protein contained in one phage particle.

To demonstrate that these amounts are found only if infection occurs, control experiments have been carried out in which radioactive fd phages were incubated with preinfected cells. Only negligible amounts of radioactive DNA and protein remained with the bacteria. This is expected since there is no super-infection of infected bacteria (Hoffmann-Berling et al., 1963).

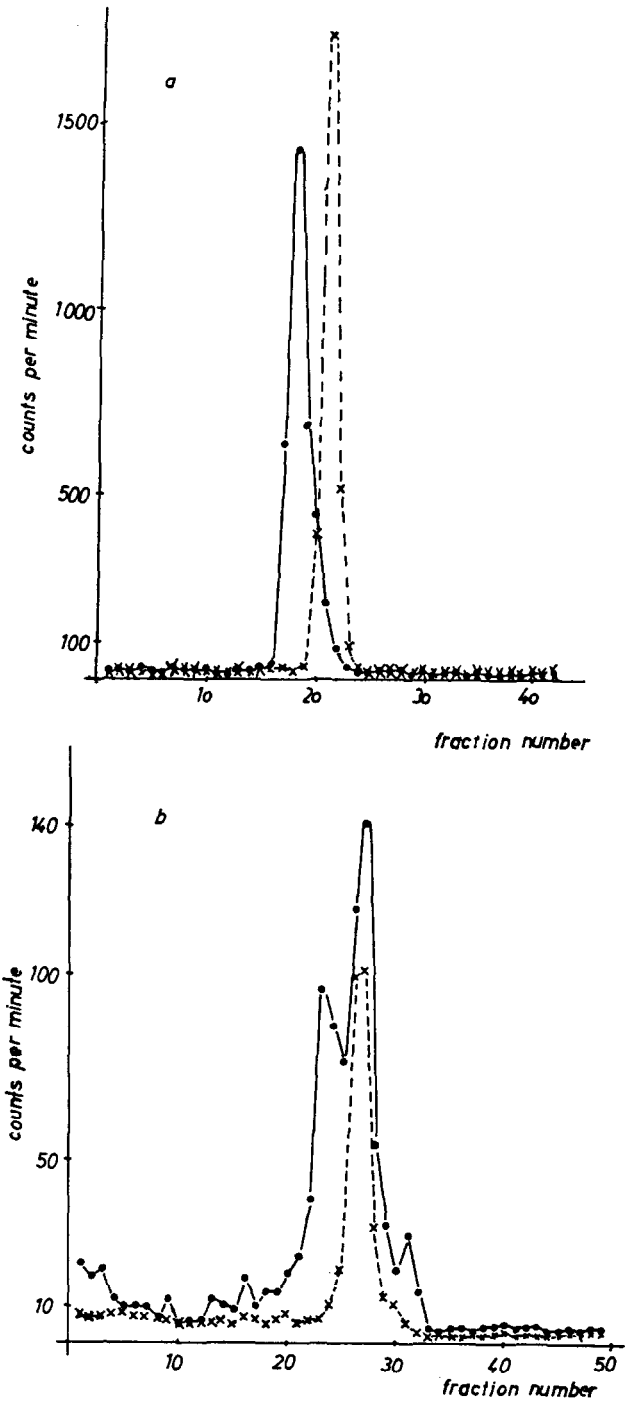

Figure 1 CsCl density centrifugation of fd phage

a. Control experiment comparing heavy (BU containing) phage with ^{14}C labelled protein (• ——— •) and light phage containing ^{32}P (× – – – – – ×).

b. Phage preparation after infection of bacteria with heavy (BU containing) phages labelled by ^{14}C in the protein.

For density experiments, bacteria were infected with phage particles containing 5-BU instead of thymine in the DNA, and protein labelled by ^{14}C amino acids. Such heavy phages are easily separated from light control phages labelled with ^{32}P in CsCl gradient (Fig. 1a).

2×10^8 bacteria/ml were incubated for 17 min. with 2×10^9 heavy radioactive phage (= 10 000 cpm) in TPG-medium containing 20γ thymine/ml and 0.025% casamino acids/ml. During this time, there is very little release of progeny phage and therefore no reinfection. The bacteria were isolated by centrifugation, suspended in medium without phage, and incubated for another 90 min. to produce and release progeny phage. Thereafter, the bacteria were removed by centrifugation. The supernatant still contains some heavy phages which have not yet invaded bacteria, and light progeny phages already produced by infected cells. The phages were isolated and centrifuged in a CsCl gradient. It was found that a considerable proportion of the radioactive protein is found in the light phage band (Fig. 1b). Therefore, protein of infecting phages has been reutilized to produce progeny phages. The total amount of radioactive protein detected in light phage, related to the number of infective centers after the primary incubation corresponds to the protein content of 0.8 - 1.1 phage particles per infective center. Control experiments show that this value is not significantly higher if the infected bacteria are incubated for 5 hr. instead of 90 min. However, it may be somewhat lower because the measurement of infective centers might not include bacteria in which part of the invading phage is still outside the cell.

4. DISCUSSION

The experiments show that most or all of the protein of the infecting phage invades the host cell, and that the protein component is reused by the infected cell to synthesize phage progeny. Invasion, in this connection, is defined as any attachment of phage protein to the host cell plasma or host cell wall that renders it inaccessible to the outside, e.g. by digesting enzymes. The most likely interpretation is that the whole infecting phage enters the host cell either with its original structure intact or with a change in the conformation of that structure. Our result is relevant to the current discussion on the possible role of F-pili in the mechanism of infection (Ippen and Valentine, 1966; Caro and Schnös, 1966; Fareed et al., 1966).

In previous investigations on phage M13, which is closely related to fd, Tzagoloff and Pratt (1964) have found very small amounts of phage protein associated with the bacteria of an infected culture. This was taken as evidence that the protein component does not enter the host cell. However, the data seem to be consistent with the opposite conclusion since we found that under the special conditions employed in these experiments only a small fraction of the bacteria is primarily infected.

It remains undecided whether the protein subunits of infecting phages are digested into amino acids to build protein both of progeny phage and of the cell, or whether the viral protein is reattached to newly synthesized DNA without prior degradation. The latter alternative seems more likely since most of the protein of infecting phages reappears in progeny viruses, while virus protein synthesis is known to comprise only a part of the total protein synthesis of the cell.

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