THE A PROTEIN OF BACTERIOPHAGE fd: ITS INTERFERENCE WITH VIRAL INFECTION

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Abstract

The A protein of bacteriophage fd has been isolated from disrupted virus by Sephadex column chromatography. After exposure to A protein, E. coli is less susceptible to virus infection. Presumably the A protein competes with intact virus for the limited number of adsorption sites on the cell surface. The results suggest that the binding of A protein to the bacterium is reversible.

Introduction

Bacteriophage fd contains two major components: a single stranded circular DNA molecule of 1.9 x 10^6 molecular weight (1), and 2,500 to 3,000 copies of the "B" coat protein, a small protein of 50 amino acid residues (2), encoded by gene 8 of the phage. A minor component, the gene 3 "A" protein is present in approximately 4 copies per virion (3). The A protein has been implicated in adsorption, and is presumed to be located at one or both ends of the filamentous virus. The isolation and some of the chemical properties of the A protein have recently been described (4).

Here we report on the biological activity of purified A protein, with respect to its competition for adsorption sites on the bacteria. The details of isolating the A protein, and its physical and chemical properties, are to be published elsewhere.

Methods

Purified fd bacteriophage was heated at 100°C for two minutes in 1% sodium dodecyl sulfate, 0.13 M dithioerythritol, 0.1 M tris·HCl buffer at pH 7. The resulting solubilized material was then run through a Sephadex G-200 column, with 0.1% SDS, 0.1 M tris buffer at pH 7 as the eluant. DNA, major protein and A protein fractions were identified and separated in accordance with their U.V. absorption characteristics. To increase the purity of the A protein, fractions from one or more column runs were combined, concentrated by rotatory evaporation, and then rerun through the same column one or more times. Incorporation of tritium labeled histidine allowed convenient identification of the A protein. Concentrations of the A protein were calculated using an experimentally determined extinction coefficient at 276 nm of 0.81/mg/ml.

Results

To demonstrate the ability of A protein to block the absorption of fd to its host bacterium the following type experiments were performed. A 6 ml overnight culture of E. coli, strain S26, was centrifuged and the bacterial pellet resuspended in 3 ml of Fraser and Jerrel's glycerol medium (5). After incubation at 37° for 10 min., the culture was divided equally into two centrifuge tubes, and 0.5 ml of A protein solution (9.5 µg/ml in 0.1 M tris buffer, pH 7) was added to one and an equal amount of tris buffer was added to the "control". 5 minutes later, 0.1 ml of fd solution (3×10^{11} PFU*/ml) was added to each tube. Both cultures were centrifuged after 5 more minutes of incubation at 37°, and after discarding the supernatant solutions, the bacterial pellets were washed twice, and each resuspended in 2.1 ml of tris buffer. The resulting suspensions were then assayed for infective centers. In other experiments anti-fd B protein serum was added to the infected cultures prior to their being pelleted and washed.

The results of experiments 1B, 2B and 3B, presented in Table I,

*PFU = plaque forming unit.

TABLE I

EFFECT OF A PROTEIN ON INFECTIVE CENTERS (PFU/ml)

Experiment 1

	A protein	Anti-B Serum	Final E.coli Concentration*	(PFU/ml)
A	-	-	3×10^9/ml	1.1×10^9
B	+	-	"	1.2×10^7
C	-	+	"	1.0×10^9
D	+	+	"	5.2×10^6

Experiment 2

	A protein	Final E.coli Concentration*	(PFU/ml)
A	-	1.5×10^9/ml	5.0×10^8
B	+	"	5.4×10^6
C	+, cells washed before adding virus.	"	3.5×10^8
D	+, virus added at same time.	"	2.8×10^7

Experiment 3

	A protein	Final E.coli Concentration**	(PFU/ml)
A	-	3.3×10^9/ml	1.8×10^9
B	+	3.3×10^9	2.8×10^7
C	+,cells washed before adding virus.	2.9×10^9	1.4×10^9
D	+, virus added at same time.	3.5×10^9	6.9×10^7

*Approximate concentration estimated by counting in a Petroff-Hausser Counting Chamber those cells visible by phase contrast microscopy.

**Determined from bacterial colony counts.

The concentration of fd used in experiments 1 and 2 was 3×10^{11} PFU/ml, while in experiment 3 it was 3×10^{13} PFU/ml.

indicate that preincubation with A protein reduces the number of infective centers, as compared to the respective controls, approximately 100 fold. When the virus was added to the bacteria at the same time as the A protein (experiments 2D and 3D), instead of 5 min. later, the number of infective centers was approximately 5% of the controls. The above results may be interpreted to mean that the A protein binds to the same sites on the E. coli cell wall which are specific for fd adsorption.

To assure that the resulting plaques arose from infected cells, and do not reflect just adsorbed and nonpenetrating virus, in experiments 1C and D anti-fd B protein serum was added to the bacteria a few minutes after infection. The results for these experiments indicate only slightly fewer PFU/ml than when anti-B serum was not employed.

In experiments performed to determine whether the A protein binds irreversibly to E. coli, the cells were washed three times prior to addition of the bacteriophage, to remove those molecules not tightly bound to the bacteria. Experiments 2C and 3C indicate that, as a result of the washing, 70% or more of the bacteria regain their susceptibility to fd infection, and suggest that the A protein - E. coli association is essentially reversible, and only partially resistant to washing. In contrast, the binding of fd to E. coli has been reported to be irreversible (6).

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