TRANSFECTION: ENHANCEMENT BY ASSEMBLY PROTEIN OF BACTERIOPHAGE R17

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Received October 3, 1975

Summary

Assembly protein was isolated by DEAE cellulose chromatography from disrupted R17 bacteriophage and reconstituted with purified R17 phage RNA. Following reconstitution, ^{125}I labeled assembly protein co-sediments with 27S R17 phage RNA in a sucrose gradient. SDS-polyacrylamide gel analysis of the 27S ^{125}I labeled protein-RNA complex confirmed that assembly protein was the only phage protein associated with the RNA. The specific infectivity (PFU/μg RNA) of the R17 phage RNA-assembly protein complex was 35-fold greater than that of R17 phage RNA when assayed on Escherichia coli spheroplasts. Infectivity of both preparations was destroyed by treatment with pancreatic ribonuclease A. Furthermore, the assembly protein-RNA complex was infectious for intact cells whereas phage RNA was not infectious. Infectivity of this 27S complex for intact cells was totally eliminated by pretreatment with ribonuclease.

Roberts and Steitz reported that reconstitution of R17 phage RNA with assembly protein resulted in a significant increase in the number of infectious units (1). This increase was attributed to the contamination of assembly protein preparations with coat protein, resulting in the subsequent reconstitution of complete infectious phage particles. In this study the reconstitution experiment has been repeated and fate of 27S phage RNA which is free of coat protein following reconstitution has been examined. Assembly protein binds to 27S phage RNA during reconstitution resulting in an increased infectivity of the RNA for spheroplasts. These preparations can also infect intact cells, supporting the concept that assembly protein functions as the phage adsorption site to susceptible cells.

Materials and Methods

The growth and purification of R17 phage has been described (2,3). Phage RNA was extracted with phenol (4) and assembly protein was purified from dissociated phage according to the method of Steitz (5). Lactoperoxidase catalyzed iodination of assembly protein was done as described by Gibsen (6). This procedure can be performed in the presence of urea (7) which is essential to keep assembly protein solubilized. The ^{125}I labeled protein was used immediately. Free ^{125}I was removed

during the subsequent dialysis step in reconstituting the protein with RNA and sucrose gradient sedimentation of the assembly protein-R17 phage RNA complex. The ^{125}I labeled protein preparations were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis. The 0.8 x 8.5 cm gels were prepared and run according to the method of Weber and Osborn (8). Gels were fractionated by cutting them into 2 mm slices and counting the fractions in a Beckman Biogamma counter.

The purified assembly protein and R17 phage RNA preparations were reconstituted essentially as described by Roberts and Steitz (1). Assembly protein (23 µg/0.4 ml) was added to R17 phage RNA (660 µg/0.2 ml). The final urea concentration was 5.3 M. As a control for subsequent infectivity studies, R17 phage RNA without added assembly protein was subjected to identical conditions for reconstitution. The mixtures for reconstitution were placed in cleaned dialysis sacs (1), and dialyzed overnight at 5 C against two changes of TSE buffer (0.15 M NaCl, 0.001 M ethylenediamine-tetracetate, 0.02 M Tris-HCl, pH 9.0) supplemented with 1% beta-mercaptoethanol and 0.01 M $MgCl_2$. The reconstituted preparation was placed on a 5-20% sucrose gradient and then centrifuged in the SW50 rotor (50,000 rpm, 2 hr, 5 C). The 27S RNA and 27S RNA-assembly protein complex containing fractions were frozen at -20 C.

The R17 phage and RNA-assembly protein complex was assayed on both *E. coli* S26 spheroplasts and cells. The spheroplast assay for infectivity of R17 phage RNA has been described (9). The infectivity of the preparations were assayed on intact *E. coli* S26 cells in the following manner. Cells were grown in 75 ml of MS medium to 1.5 x 10^8 cells/ml, divided into three cultures, pelleted and resuspended in 0.5 ml of MS medium. The cultures received 0.1 ml R17 RNA, 0.1 ml R17 RNA-assembly protein complex or 0.1 ml of the complex pretreated with 10 µg of pancreatic ribonuclease A for 15 min at 37 C. The inoculated cultures were incubated for 1 min at 37 C and then 20 min at 22 C. A 0.1 ml fraction of each culture was removed and the infected cells assayed directly by the standard agar overlay plaque technique (9). The remaining 0.4 ml of each culture was added to 50 ml of MS medium and incubated for 3 hr at 37 C. The cells were harvested by shaking with 5% chloroform (v/v) to release cell-associated phage and the phage titer assayed by the plaque method.

Results

The assmebly protein fraction was iodinated with ^{125}I by the lactoperoxidase method and analyzed in 10% polyacrylamide gels containing 0.1% SDS (Fig. 1). Although the assembly protein elutes from the DEAE chromatography column at a considerable distance from coat protein fractions, gel electrophoresis indicates it is contaminated with coat protein. Some coat protein may be retained by the DEAE cellulose and co-elute with assembly protein because of incomplete dissociation of the coat and assembly capsid proteins. Preparations eluting from columns with the highest ratio of assembly protein to coat protein were most effective in these reconstitution studies. This may reflect a larger input of assembly protein per µg of protein added to the reconstitution mixture or perhaps the proposed aggregates of assembly and coat protein do not reconstitute with phage RNA.

Assembly protein eluted from a DEAE cellulose column was iodinated with ^{125}I,

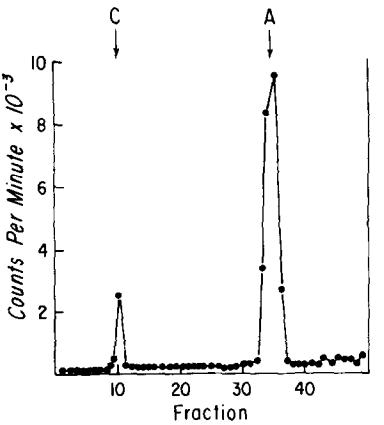

Fig. 1. Polyacrylamide gel electrophoresis of lactoperoxidase ^{125}I labeled protein from the assembly protein fraction of a DEAE cellulose column. The column fractions containing assembly protein were iodinated with ^{125}I by the lactoperoxidase method. Lactoperoxidase-bound beads were removed by low speed centrifugation and unbound iodine was removed by dialysis against 8 M urea. The labeled protein was briefly heated to 90 C in 2% SDS and run on 8.5 cm 10% polyacrylamide gels containing 0.1% SDS as previously described (8). Purified R17 phage was run on a parallel gel and proteins stained with Coomassie brilliant blue. C and A represent the position of the stained coat and assembly proteins, respectively. Migration is from right to left.

mixed with R17 RNA and reconstituted. The reconstituted mixture was sedimented through a 5 to 20 percent sucrose gradient and then the RNA and labeled protein distribution was determined (Fig. 2). The major portion of labeled protein is found in the pellet and the top of the sucrose gradient. However, a distinct fraction of labeled protein co-sediments with the R17 phage RNA. This co-sedimentation appears to represent a specific RNA-protein interaction, since the labeled protein associated with the 27S RNA is totally composed of assembly protein (Fig. 3). If the co-sedimentation of protein with the RNA was non-specific, the coat protein contaminents of assembly protein preparations should also sediment with the RNA. The coat protein remains near the top of the sucrose gradient. Assembly protein which was stored for 72 hr in the column elution buffer prior to iodination and reconstitution with R17 RNA no longer co-sediments with the 27S RNA.

The effect of binding of assembly protein to R17 RNA on the infectivity of the protein-RNA complex was examined. For comparative assays of infectivity, R17 RNA and assembly protein-R17 RNA mixtures were exposed to the same reconstitution

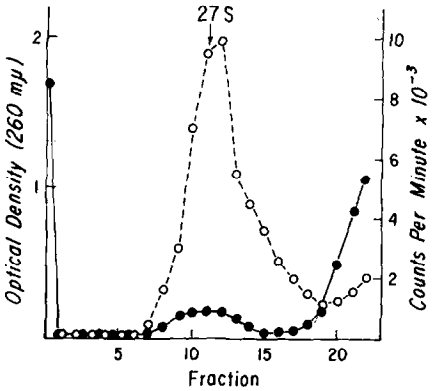

Fig. 2. Velocity sedimentation of a reconstituted mixture of R17 RNA and assembly protein. R17 phage RNA (660 μg/0.2 ml) was added to ^{125}I labeled assembly protein (16 μg/0.4 ml) and reconstituted as described in Materials and Methods. A 0.3 ml portion of the reconstituted mixture was centrifuged in a 4.4 ml 5 to 20% sucrose gradient (50,000 RPM; 2 hr, 5 C). Sedimentation is from right to left. o--o optical density of R17 RNA, ●--● ^{125}I labeled assembly reconstituted with R17 RNA.

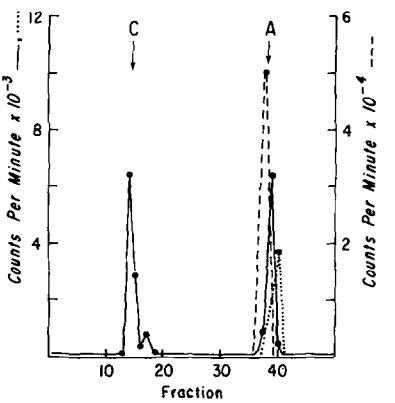

Fig. 3. Polyacrylamide acrylamide gel analysis of a reconstitution reaction mixture. R17 RNA was reconstituted with ^{125}I labeled assembly protein and centrifuged in a 5 to 20% sucrose gradient as shown in Fig. 2. The labeled protein in the pellet, 27S region and top of the gradient were briefly heated in 2% SDS and run on 10% polyacrylamide gels containing 0.1% SDS. Purified R17 phage was solubilized in 2% SDS and run on a parallel gel. The C and A markers represent the positions of the stained coat and assembly protein bands, respectively. Migration was from right to left. ___ labeled protein at the top of the sucrose gradient, ···· 27S region of the gradient, and --- pellet.

procedures. Following reconstitution, samples were sedimented through 5 to 20% sucrose gradients and the specific infectivity (PFU/μg RNA) of the 27S fractions were compared. When infectivity was assayed on E. coli spheroplasts the specific

TABLE I

Effect of assembly protein on infection of spheroplasts with R17 phage RNA

Exp.	Reconstituted R17 RNA	Reconstituted A protein	RNase	Specific infectivity (PFU/μg RNA)
1A	+	-	-	3.3×10^3
1B	+	-	+	0
1C	+	+	-	1.7×10^4
1D	+	+	+	0
2A	+	-	-	6.6×10^2
2B	+	+	-	1.7×10^4
3A	+	-	-	2.2×10^4
3B	+	+	-	7.7×10^5

All of the RNA-containing samples were isolated from the 27S region of sucrose gradients following reconstitution. A portion of each RNA and reconstituted RNA-A protein mixture was treated with 10 μg of pancreatic ribonuclease for 15 min at 37 C and then assayed for infectivity. The numbers 1, 2 and 3 refer to independent experiments using different lots of R17 RNA and A protein.

infectivity of the R17 RNA-assembly protein complex was 5 to 35 fold greater than R17 phage RNA (Table 1). It is unlikely that the increase in infectivity is due to contamination of the RNA with reconstituted phage particles, since the sample was obtained from the 27S region of the gradient, is RNase sensitive, and no coat protein is found in this region of the gradient (Fig. 3). Thus it appears that assembly protein enhances infectivity of R17 RNA for E. coli spheroplasts.

Since assembly protein has been implicated in attachment of RNA phage to cells, it was of interest to determine if intact E. coli cells could be infected with the R17 RNA-assembly protein complex. Cells were exposed to either R17 RNA or the R17 RNA-assembly protein complex and then incubated for 3 hr to allow for phage replication (Table 2). Although R17 RNA was not infectious for intact E. coli cells, a significant yield of R17 phage was obtained from cells infected with the 27S RNA-

TABLE II

Effect of assembly protein on infection of Escherichia coli cells with R17 phage RNA.

Exp.	Reconstituted R17 RNA	Reconstituted A protein	RNase	Resulting plaque forming units/ml
1A	+	-	-	0
1B	+	+	-	2.8×10^4
1C	+	+	+	0
2A	+	-	-	0
2B	+	+	-	2.3×10^3
3A	+	-	-	0
3B	+	+	-	9.1×10^4

The numbers 1, 2 and 3 represent independent experiments using different lots of R17 RNA and A protein. The RNA-containing samples were obtained from the 27S region of a sucrose gradient and in some instances treated with RNase as described in Table I.

assembly protein complex. Infectivity of the complex was completely inhibited by treatment with RNase prior to infection and the phage produced was neutralized by R17 antisera. The above assay which allows for several cycles of replication was used because no infectious centers were observed when cells were substituted for spheroplasts in the infectious center assay shown in Table 1. This probably reflects a much lower efficiency of infection of cells by the complex, since most of the RNA is not bound to assembly protein (Fig. 2) and would not be infectious for intact cells.

Discussion

The experiments in this paper were begun to determine if assembly protein assists in the entrance of phage RNA into cells. Studies with amber mutants have indirectly implicated assembly protein in adsorption of phage to F pili by showing

that mutants lacking assembly protein do not absorb (10,11). These studies are supported by the report that assembly protein is a surface protein of the phage and therefore accessible as a possible adsorption site (12). The data presented in this study indicate that assembly protein can bind to R17 RNA without binding of coat protein resulting in an enhancement of the infectivity of the RNA. Not only are spheroplasts more sensitive to the R17 RNA-assembly protein complex but intact cells which are totally resistant to infection with deproteinized R17 RNA can be infected with the RNA-assembly protein complex. The enhancement of infectivity may be due to absorption of the RNA to cells or the penetration of the RNA into the cell. Since the phage adsorbs to the F pilus (13) it is assumed that the RNA enters through the F pilus or is transmitted down the surface of the F pilus for entry into the cell at the plasma membrane. The ability of the R17 RNA-assembly protein complex to infect intact cells may reflect the ability of the RNA to attach to the F pilus absorption site for transport into the cell. Since the assembly protein is transported into the cell with the RNA rather than remaining attached to the absorption site (14,15), it may also be important in the RNA transport mechanism.

A definite enhancement of the infectivity of R17 RNA was noted even though the efficiency of binding assembly protein to the RNA was low. Reconstitution of the components of RNA phage particles is also characterized by a low efficiency and the probable reasons for the low yield have been reviewed (16).

A small amount of coat protein is found in the assembly protein fraction from DEAE columns. Ordinarily free coat protein would bind to the R17 phage RNA during reconstitution (17). In these studies, however, the binding of the coat protein contaminant to the R17 RNA did not occur (Fig. 4). The coat protein contaminant in the assembly protein preparations may be bound to assembly protein which could account for its retention by the DEAE resin and co-elution with assembly protein. A coat protein-assembly protein antigenic specificity has been demonstrated by immunodiffusion of R17 antiserum against dissociated phage protein (W. Iglewski, submitted J. Virol.). A preformed aggregate of coat and assembly protein may not be able to bind to R17 RNA.

The inability of assembly protein deficient mutants of RNA phage to adsorb to the F pilus of E. coli has provided indirect evidence for the role of this protein as the phage adsorption site (10,11). The data presented in this paper further support and extend this concept. The existence of a complex between the phage genome and the cellular attachment site would favor entrance of the genome into the cell following dissociation of the coat protein capsid.

Acknowledgements

The author is grateful to D. Ohman for technical assistance and the Medical Research Foundation of Oregon for support.

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