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Adsorption Protein of Bacteriophage fl: Solubilization in Deoxycholate and Localization in the fl Virion†

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ABSTRACT: A complex containing the minor coat protein or adsorption protein (A protein) of bacteriophage fl has been solubilized from the fl virion, using the detergent deoxycholate. This complex was resolved from the fl DNA and from the fl major coat protein, or B protein, by gel filtration in the presence of deoxycholate. The A protein complex migrated as a single band on sodium dodecyl sulfate-urea-polyacrylamide gels corresponding to a molecular weight of 60 000. Analysis of the amino acid composition and amino terminal residues of this preparation indicates that the preparation contains a 20% contamination of additional protein species. Antibody against purified fd A protein is cross-reactive with deoxycholate-purified fl A protein and with fl phage. Electron microscopic observation of negatively stained complexes of fl phage with this anti-fd A protein antibody and ferritin conjugated goat anti-rabbit IgG antibody revealed phages with ferritin particles at their termini or complexes of two or more phages joined together at one end by ferritin, indicating that the complex of A protein molecules is located at one end of the filamentous fl virion.

The closely related filamentous bacteriophages fl, fd, and M13 are flexible rod-shaped particles approximately 8500-9000 Å long and 60-70 Å wide which infect male (F^+) *Escherichia coli* (Marvin and Hohn, 1969). They consist of a single-stranded-circular DNA molecule ensheathed by approximately 2400 molecules of the major coat protein or B protein, encoded by gene 8, and by several molecules of the minor coat protein or adsorption (A) protein,[1] specified by gene 3. The filamentous phages have proven to be most useful model

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[1] Abbreviations used are: A protein, the minor coat protein or absorption protein; B protein, the major coat protein; RF, double-stranded replicative form DNA; IgG, immunoglobulin; TE buffer, 0.01 M Tris, 0.001 M EDTA, pH 7.8; DOC, deoxycholate; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; EDTA, (ethylenedinitrilo)tetraacetic acid; BSA, bovine serum albumin; DEAE, diethylaminoethyl.

systems for studying DNA synthesis (Schekman et al., 1974) and RNA and protein synthesis (Model and Zinder, 1974), as well as for investigating viral morphogenesis and the structure and assembly of multisubunit macromolecules (Marvin and Wachtel, 1975). The major coat protein has been investigated extensively and is proving to be an intriguing model membrane protein (Woolford et al. 1974; Makino et al., 1975; Woolford and Webster, 1975; Wickner, 1976).

However, only limited data exists concerning the structure or function of the adsorption protein. Studies on the A protein have suggested that this protein is located only at one end of the phage and is important in maintaining the structure of the phage (Caro and Schnos, 1966; Fareed et al., 1966; Pratt et al., 1969; Rossomando and Zinder, 1968; Rossomando and Milstein, 1971; Ikehara and Utiyama, 1975; Marco, 1975). The A protein has been implicated in the adsorption of the phage to the F pili of host cells (Caro and Schnos, 1966; Pratt et al., 1969) and upon binding and/or eclipse of the phage the A protein is altered to a lower molecular weight form (Marco et al., 1974). The adsorption has been isolated in a complex with the replicative form DNA attached to the inner (cytoplasmic) membrane of the host cell and appears necessary for the conversion of the phage DNA to the double-stranded replicative form (RF) (Jazwinski et al., 1973). It has been suggested that this protein may act as a "pilot protein" to guide the infecting phage and its DNA into the cell and through the inner membrane, and to incorporate the phage DNA into the ongoing cellular machinery of DNA replication (Kornberg, 1974). Analogous "pilot proteins" have been found in the DNA bacteriophages S13 and ϕX174 (Jazwinski et al., 1975a,b) and in the RNA bacteriophages (Krahn et al., 1972). The adsorption protein of the filamentous phages is therefore of interest from a structural and functional point of view, since it appears to interact with such diverse biological structures as the phage DNA, the inner membrane of the host bacteria, the replication machinery of the host cell, the bacterial "phage receptors", and probably the major coat protein of the phage.

We attempted isolation of the A protein in as native a configuration as possible by solubilizing the virion with deoxycholate, a detergent that does not destroy the structure or biological activity of many proteins (Makino et al., 1973). A complex containing the A protein was subsequently fractionated from the fl DNA and B protein by gel filtration in the presence of deoxycholate. The molecular weight, amino acid composition, antigenic properties, and the number of A proteins per phage were determined. Compared with similar results for the A protein of bacteriophage fd (reported in the preceding paper of this issue, Goldsmith and Konigsberg, 1977), these data suggest that the A proteins from fl and fd are similar in many respects, although possibly different in their detailed chemical structure.

Experimental Procedures

Materials. S26Rle (K37), a serine inserting suppressing (Su^+) strain of *E. coli* K12, was used (Weigert and Garen, 1965). The fl phages were obtained from Dr. Norton Zinder of Rockefeller University and the fd phages were a generous gift of Dr. William Konigsberg. Polyethlene glycol (Carbowax 6000) was purchased from Union Carbide. Deoxycholate was purchased as the sodium salt from Fisher Scientific Co. and was recrystallized from acetone as described in Makino et al. (1975). Sephadex G-25, Sephadex G-150, Sepharose 4B, and blue dextran 2000 were obtained from Pharmacia. Lauryl sodium sulfate, L-amino acids, and L-[G-^{3}H]lysine (7 Ci/mmol) were purchased from Schwarz/Mann. L-[3-^{3}H]Histidine (8.4 Ci/mmol), L-[3-^{3}H]arginine (28.7 Ci/mmol), L-[U-^{14}C]lysine (306 mCi/mmol), L-[4,5,N-^{3}H]leucine (60 Ci/mmol), L-[3,5-^{3}H]tyrosine (53.6 Ci/mmol), and Aquasol-2 were bought from New England Nuclear. Acrylamide and bisacrylamide were purchased from Eastman Kodak. Coomassie brilliant blue R was a product of Sigma. Diethylaminoethylcellulose (DE-52) was obtained from Whatman. Goat antiserum against rabbit IgG was a product of Miles Laboratories, Inc. Ferritin was obtained from Pentex, Inc., and Bio-Gel A-5m, 200–400 mesh, from Bio-Rad Laboratories.

Solubilization and Separation of the A Protein Complex. Unlabeled and radioactively labeled fl phages were prepared as described in Makino et al. (1975). The phage proteins were solubilized and separated using the procedure of Makino et al. (1975) with the following differences. The proteins were solubilized from approximately 30 mg of phage by incubation at 37 °C for 1 h in 4 mL 0.01 M Tris, 0.001 M EDTA, 0.06 M deoxycholate (DOC), pH 8.5, and 50 μL of chloroform. The chloroform that remained was evaporated with nitrogen and the sample layered on a gel filtration column was composed of 600 mL of Sephadex G-150 layered on top of 300 mL of Sepharose 4B, both equilibrated with 0.01 M Tris, 0.001 M EDTA, and 0.03 M DOC, pH 8.5. Fractions corresponding to A protein were pooled from the large Sephadex–Sepharose column, making sure to avoid contamination of A protein by fl DNA or B protein. These fractions were dialyzed for 12 h against 4 L of 0.01 M Tris, 0.001 M EDTA, pH 8.5, with two changes, then against 4 L of deionized water for approximately 12 h each with two to three changes. This 50–60-mL sample was then rotoevaporated to about 10 mL and dialyzed again against about 2 L of 0.01 M Tris, 0.001 M EDTA, pH 8.5, with two changes, and then against 2 L of deionized water with two changes. This was then lyophilized and stored at −20 °C.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Approximately 100 μg of the lyophilized A protein was resuspended in 0.3 mL of 0.01 M Na_2HPO_4, pH 7.2, containing 15% glycerol, 2 M urea, 1% sodium dodecyl sulfate, and 2% β-mercaptoethanol, incubated at 90 °C for approximately 50 min, and electrophoresed on 8% polyacrylamide gels as described by Webster and Cashman (1973).

In order to determine the molecular weight of the A protein, 10–20 μg each of proteins of known molecular weight (Weber and Osborn, 1969) were electrophoresed with purified A protein. The standard proteins used and their molecular weights were transferrin, 78 000; catalase, 60 000; ovalbumin, 43 000; chymotrypsinogen, 25 600; and lysozyme, 14 300.

Amino Acid Analyses. Amino acid compositions were only determined for samples of lyophilized A protein which yielded a single 60 000 molecular weight band on polyacrylamide gel electrophoresis (Spackman et al., 1958).

Amino-Terminal Determination. The amino-terminal analysis was performed by Dr. William Konigsberg (Goldsmith and Konigsberg, 1977).

Isolation of Antibodies and Conjugation with Ferritin. Rabbit antiserum against purified fd A protein was kindly provided by Drs. Merril Goldsmith and William Konigsberg. The IgG fraction from this serum and from the goat anti-rabbit IgG serum was purified by precipitation with 40% saturated ammonium sulfate three times at 4 °C, followed by chromatography on a 1.5 × 25 cm column of diethylaminoethylcellulose equilibrated with 0.02 M KH_2PO_4, pH 8.0. The IgG fractions were pooled, concentrated by ultrafiltration with an Amicon PM30 ultrafiltration dialysis membrane, and stored frozen at −20 °C until use. Control rabbit IgG directed against

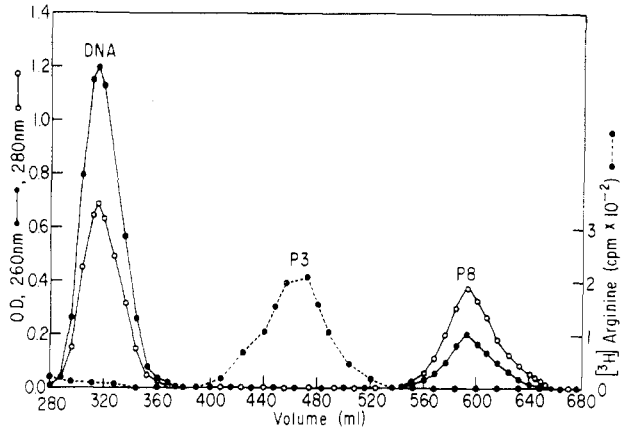

FIGURE 1: Gel chromatographic purification of fl A protein. Tritiated arginine-labeled fl (30–60 μg) plus approximately 40 mg of unlabeled fl phage were solubilized in 0.01 M Tris, 0.001 M EDTA, 0.06 M deoxycholate, pH 7.8, with chloroform at 37 °C for 1 h. The chloroform was evaporated with nitrogen and the sample was layered on the column equilibrated with 0.01 M Tris, 0.001 M EDTA, 0.03 M deoxycholate, pH 8.5, and was eluted with the same buffer. The column consisted of approximately 600 mL of Sephadex G-150 layered on top of 300 mL of Sepharose 4B, so that the 60 000 molecular weight A protein could be resolved from the 2×10^6 molecular weight fl DNA as well as from DOC micelles containing a dimer of the 5240 molecular weight fl B protein (i.e., 16 000 molecular weight including DOC). (● - - - ●) Counts per minute of [^{3}H]arginine; (●–●) absorbance at 260 nm; (○–○) absorbance at 280 nm. P3 refers to the A protein, P8 the major coat protein.

rat liver sulfite oxidase was a kind gift of William Southerland of this department. Purified goat anti-rabbit IgG was conjugated with ferritin by the method of Olsen and Prockop (1974). Ferritin-labeled goat antibodies were stored at −20 °C and used within 2 weeks of their synthesis.

Reaction of the A Protein Complex with Anti-fd A Protein Antibody. The isolated A protein complex was reacted with antibody by adding 1 mL of A protein to 4 mL of buffer A (0.1 M Tris, pH 7.64, 0.05 M NaCl, 1 mg/mL BSA), and then adding 0.03 mg of either anti-fd A protein antibody or anti-sulfite oxidase antibody. The antibody (IgG) was purified from serum by ammonium sulfate precipitation and DEAE chromatography, as described above. After incubation at 37 °C for 4 h, 1.2 mg of goat anti-rabbit IgG in 1 mL of 0.01 M KPO_4, pH 7.0, was added, and the incubation was continued for 2 h at room temperature and then overnight at 4 °C. The fine precipitate which resulted was resuspended, and the radioactivity in 2 mL of this solution was measured in 20 mL of Aquasol-2 in order to determine the initial amount of A protein present. The remaining 4 mL was centrifuged in polyallomer tubes for 10 min at 25 000 rpm in a Beckman SW56 rotor. After gently pouring off the supernatant, the pellet was solubilized by the addition of 0.05 mL of H_2O and 0.5 mL of Protosol and incubation for 3 h at room temperature. Twenty milliliters of toluene-based fluid was added and the radioactivity was determined. One milliliter of buffer A was added to the 4 mL of supernatant, this solution was reacted with additional aliquots of antibody and centrifuged as above, and the amount of radioactivity was determined in the resulting supernatant and pellet. The amount of radioactivity which was precipitated or remained in the supernatant was calculated from these multiple reactions, taking into account the different counting efficiencies in the different solvents.

Formation and Isolation of Phage–Antibody Complexes. Complexes of antibody with the phage were formed as follows: fl phages (10 μg) were mixed with 0.2–1.2 mg of anti-fd A protein IgG in TE buffer to a final volume of 350 μL and incubated at 37 °C for 1 h. This solution was then layered on 3.45 mL of 7.5% sucrose, 0.01 M Tris-HCl, pH 7.8, and centrifuged at 50 000 rpm (246 000g) for 3 h in a Beckman SW56 rotor. The supernatant, containing unreacted antibody, was carefully removed and the pellet of phage–antibody complex was resuspended in 50 μL of 0.01 M Tris-HCl, pH 7.8, at 4 °C for 16 h. The complex was then incubated with 100 μL of the ferritin-conjugated goat anti-rabbit IgG (~150 μg of IgG) plus 200 μL of 0.01 M Tris-HCl, pH 7.8, at 37 °C for 1 h, and was centrifuged as described above. The resulting pellet containing a complex of fl phage, rabbit and A protein, and goat anti-rabbit IgG was resuspended in 50 μL of 0.01 M Tris-HCl, pH 7.8. As a control, rabbit IgG directed against liver sulfite oxidase was used in place of anti-A protein IgG in the above procedure.

Electron Microscopic Observation of Phage–Antibody Complexes. The solution (50 μL) containing the phage–antibody complex was diluted into 0.5 mL of 0.1 M ammonium acetate pH 7.0. A 200- or 400-mesh formvar-coated copper grid was floated on a droplet of this solution for 30 s, rinsed in ammonium acetate, and then negatively stained with 2% uranyl acetate in H_2O for 1 min. The samples were viewed at 80 kV in a JEOL 100C electron microscope and photographed at a magnification of 25 000–30 000. A series of 15 adjacent fields containing phage was photographed for each sample for further study and statistical analysis.

Results

Isolation of Fl A Protein. Although the adsorption protein is very much larger than the major coat (B) protein (60 000 vs. 5240 daltons), it has previously only been resolved from the B protein by disruption of the phage with NaOH or the denaturing detergent sodium dodecyl sulfate, followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis or sucrose gradient velocity sedimentation and gel filtration column chromatography in the presence of sodium dodecyl sulfate (Rossomando and Zinder, 1968; Henry and Pratt, 1969; Webster and Cashman, 1973; Jazwinski et al., 1973; Marco et al., 1974; Marco, 1975; Makino et al., 1975; Segawa et al., 1975). However, we have found that deoxycholate, a detergent that does not destroy the biological activity of many proteins (Makino et al., 1973), will solubilize both the major and minor coat proteins of fl (Makino et al., 1975).

The proteins can be separated by gel filtration on a double-headed Sephadex G-150–Sepharose 4B gel filtration column as shown by the elution profile in Figure 1. The peak labeled with [^{3}H]arginine presumably corresponds to the A protein, since the B protein lacks arginine. Since the A protein constitutes only 1–2% of the total phage protein, it does not contribute a measurable amount of absorption at 260 or 280 nm. The 1.95×10^6 dalton fl DNA eluted in the void volume with an optical density 260/280 ratio of 1.8 and the B protein eluted from 560 to 640 mL with an absorbance peak of protein (280/260 = 2.2). Similar results were obtained with [^{3}H]histidine-labeled fl phage. Chromatography of solubilized phage labeled with amino acids which are present in the B protein as well as the A protein resulted in two peaks of radioactivity, a minor one at the position of the A protein and a major one at the position of the B protein.

With several independent preparations of [^{3}H]arginine-labeled fl, a second peak of [^{3}H]arginine was consistently observed eluting at approximately 620–640 mL, slightly slower than the B protein (not shown in Figure 1). This may correspond to a second minor coat protein described by Henry and Pratt (1969) and Kornberg (1974), which has a molecular

TABLE I: Reaction of Anti-A Protein Antibody with Purified A Protein.

Sample[a]	Antibody[b]	cpm supernatant[c]	cpm pellet[c]
30 mM DOC	Anti-A	170	678
	Anti-SO	640	51
0.2 mM DOC	Anti-A	283	756
	Anti-SO	980	105

[a] The [^{3}H]tyrosine-labeled A protein samples were pooled from the column in 30 mM DOC, 0.01 M Tris, 0.001 M EDTA, pH 8.5, and used as is, or else the DOC was diluted to 0.2 mM by dilution in 0.01 M Tris, 0.001 M EDTA, pH 8.5, followed by ultrafiltration to the original volume. [b] Anti-A is DEAE purified IgG against fd A protein, and anti-SO is DEAE purified IgG against sulfite oxidase. The amount of reaction was determined using goat anti-rabbit IgG as described under Experimental Procedures. [c] The sum of the radioactivity in the supernatant and pellet accounted for all of the radioactivity in the initial experiment.

TABLE II: Amino Acid Composition of fl A Protein, Residues/60 000 Daltons.

Amino acid	DOC-purified protein[a]	Sodium dodecyl sulfate purified protein[b]
Lys	33.2 ± 1.7	22.1
His	7.2 ± 1.3	2.7
Arg	12.4 ± 2.2	14.4
Asp	61.7 ± 4.1	79.0
Thr	35.1 ± 4.6	36.0
Ser	72.7 ± 2.0	38.4
Glu	71.4 ± 1.5	61.7
Pro	32.0 ± 5.4	36.4
Gly	109.3 ± 12.4	96.9
Ala	51.3 ± 3.0	43.4
Cys	3.0 ± 0.5	5.1
Val	18.3 ± 2.3	30.3
Met	4.8 ± 0.5	10.8
Ile	12.5 ± 2.1	15.5
Leu	25.7 ± 3.4	27.5
Tyr	17.5 ± 3.4	25.6
Phe	18.9 ± 1.0	30.4
Trp[c]	2.0 ± 0.4	

[a] Purified by solubilization in 60 mM (2.48%) deoxycholate and gel filtration in 30 mM deoxycholate as described under Experimental Procedures. Average of three 24-h hydrolyses using three separate samples. [b] Purified by solubilization of fl phage in 5% sodium dodecyl sulfate and 1% mercaptoethanol and subsequent gel filtration in 1% sodium dodecyl sulfate and 0.1% mercaptoethanol (Goldsmith and Konigsberg, 1977). The fl phages were prepared as described under Experimental Procedures. [c] Determined by a separate alkaline hydrolysis relative to His.

weight of approximately 3000, contains cysteine and arginine, and may be present in 25–30 copies in the phage particle. We have previously observed in sodium dodecyl sulfate–polyacrylamide gels of [^{3}H]histidine fl phage a peak of radioactivity migrating slightly faster than the B protein (Makino et al., 1975). Efforts are underway to better resolve this protein from the B protein.

Two independent criteria, sodium dodecyl sulfate–urea–polyacrylamide gel electrophoresis and immunoprecipitation

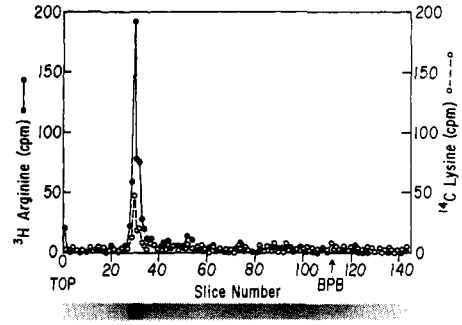

FIGURE 2: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of fl A protein isolated by column chromatography. Fl A protein was purified from [^{3}H]arginine and [^{14}C]lysine-labeled fl phage plus unlabeled carrier fl by gel filtration in the presence of deoxycholate as described under Experimental Procedures and Figure 1. Fractions corresponding to A protein were pooled and dialyzed against 0.01 M Tris, 0.001 M EDTA, pH 8.5, and then against water. The sample was lyophilized and resuspended in 0.01 M Na_2HPO_4, pH 7.2, containing 2 M urea, 1% sodium dodecyl sulfate and 2% β-mercaptoethanol, heated at 90 °C for 50 min and electrophoresed on an 8% polyacrylamide gel containing 0.1% sodium dodecyl sulfate, and 8 M urea. The gel was stained with Coomassie blue and photographed, and was then frozen and sliced into 1-mm segments. Radioactivity was measured as described in Woolford et al. (1974). BPB indicates the center of the bromophenyl blue tracking dye. (●–●) Counts per minute of [^{3}H]arginine; (○ - - - ○) counts per minute of [^{14}C]lysine.

with anti-fd A protein antibody, demonstrated that the above procedure resulted in an enrichment of the fl A protein. When the presumptive A protein complex labeled with [^{14}C]lysine and [^{3}H]arginine was subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and urea, there was one peak of radioactivity which was coincident with the one major Coomassie blue stained band of protein (Figure 2). A protein from sodium dodecyl sulfate solubilized radioactively labeled fl phage migrated at the same position (data not shown; Makino et al., 1975). Less than 5% of the ^{3}H and little, if any, ^{14}C radioactivity remained at the top of the gel, indicating that very little aggregation of A protein occurred. Furthermore, no radioactivity was present near the dye front, where the fl B protein migrates with an apparent molecular weight of 59 600 ± 3000 (eight separate determinations), in agreement with the findings of Segawa et al. (1975) and Goldsmith and Konigsberg (1977).

Antibody against fd A protein purified by the method of Goldsmith and Konigsberg (1977) was cross-reactive with the complex of fl A protein isolated as described above. Purified anti-fd IgG antibody was incubated with [^{3}H]leucine- or [^{3}H]tyrosine-labeled fl A protein pooled from the deoxycholate column. Goat anti-rabbit IgG antibody was subsequently added to precipitate the complex of A protein and anti-A protein antibody. By this method, 75% of the labeled protein was precipitated in the presence of DOC at concentrations both near and far below the critical micelle concentration (Table I). In the control experiment in which a nonspecific rabbit IgG (anti-rat liver sulfite oxidase antibody) was substituted for anti-fd A protein antibody, less than 7% of the radioactivity was precipitated.

Purity of the A Protein in the Isolated Complex. In order to determine the relative purity of fl A protein in the complex prepared using deoxycholate, both the amino acid composition and the amino terminal residues in the isolated complex were determined. As shown in Table II, the adsorption protein complex contains all amino acids, including those which are missing from the major coat protein (histidine, arginine, and cysteine). Approximately one-half of the residues differ sig-

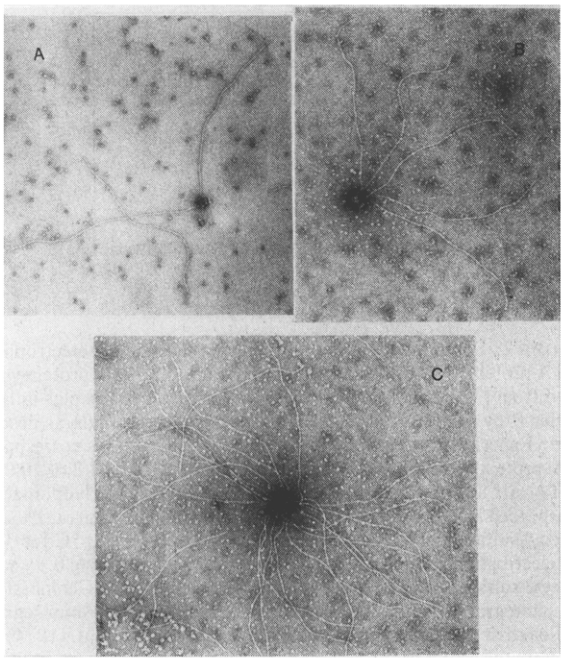

FIGURE 3: Complexes of fl phage–rabbit antibody against fd A protein and ferritin-conjugated goat anti-rabbit IgG. Fl phages were incubated with anti-A protein, purified by centrifugation, incubated with ferritin-labeled goat anti-IgG, repurified, and negatively stained, as described under Experimental Procedures. Photographs were taken at a magnification of 10 000 using a JEOL 100C electron microscope. (A) Fl phage (100 μg) plus 50 μg of anti-A protein + 150 μg of ferritin–goat anti-IgG. A doublet of phage is shown. (B) Fl phage (10 μg) plus 100 μg of anti-A protein plus 150 μg of ferritin–goat anti-IgG. A small complex of five phages connected by ferritin antibodies is shown. (C) Fl phage (10 μg) plus 200 μg of anti-A protein plus 150 μg of ferritin goat anti-IgG. A large rosette of phage is shown.

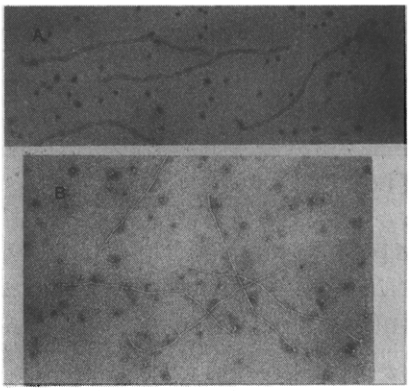

FIGURE 4: Control experiments without antibody against A protein. Incubation and purification of phage and antibodies were the same as in Figure 3. No complexes of phage with antibody were observed in samples (Table III), where antibody against A protein was replaced with a non-specific antibody (anti-rat liver sulfate oxidase) (A), or was excluded entirely (B). (A) Fl phage (10 μg) plus 200 μg of anti-rat liver sulfite oxidase plus 150 μg of ferritin goat anti-rabbit antibody. (B) Fl phage (10 μg) plus 150 μg of ferritin goat anti-rabbit antibody.

nificantly from the composition of the fd A protein, reported by Goldsmith and Konigsberg (1977). In order to rule out the possibility that the A protein of fl is different from the A protein of fd, the following experiment was performed. Goldsmith and Konigsberg prepared A protein by their method, using sodium dodecyl sulfate and mercaptoethanol, from fl phage prepared in our laboratory. We determined the composition of this material and found it to be similar to the composition of the fd A protein described by Goldsmith and Konigsberg (1977) (Table II). Thus, the difference in composition between the DOC-isolated complex and sodium dodecyl sulfate purified protein must be attributed to the separation of one or more proteins from the A protein in the presence of sodium dodecyl sulfate and mercaptoethanol that are not resolved using only DOC. This was confirmed by determining the amino-terminal residues present in the DOC-purified f1 A protein complex. Such an analysis showed that 80% of the amino-terminal residues was alanine, as expected for the A protein (Goldsmith and Konigsberg, 1977). Valine (15%) and leucine (5%) accounted for the remainder of the amino termini. These results suggest that 20% of the DOC-isolated molecules are represented by species of protein other than fl A protein.

The composition of our isolated A protein complex cannot be explained by its being contaminated solely with the fl B protein, since it does not contain an excess of valine or isoleucine, two amino acids that are present in relatively large amounts in the B protein (Asbeck et al., 1969; Nakashima and Konigsberg, 1974). The additional species of protein also do not appear to be one of several major bacterial structural proteins, such as F^- pilin, flagellin, or the four major proteins of the *E. coli* outer membrane, based on the published amino acid compositions of these proteins (Brinton, 1971; Martinez et al., 1967; Garten et al., 1975).

Structure of the A Protein Complex in the fl Virion. A variety of indirect methods has indicated that there are only a few A protein molecules per phage (Pratt et al., 1969; Scott and Zinder, 1967; Henry and Pratt, 1969; Wiseman et al., 1972). In agreement with these groups, we calculated that there are approximately 3 ± 0.4 molecules of the A protein complex present per phage based on the ratio of leucine in the A complex and B protein peaks eluting from the gel-filtration column in five independent experiments. Leucine was used, since this amino acid is present in the same amount in both the deoxycholate and sodium dodecyl sulfate purified A protein (Table II). It should be emphasized that such a calculation is only approximate, since it is based on a 60 000 molecular weight for the A protein (Segawa et al., 1975; Goldsmith and Konigsberg, 1977; this paper) and the assumption that there are 2400 molecules of the B protein per phage (Berkowitz and Day, 1976), two values which might not be accurate. The B protein has been shown to contain two leucines per molecule (Asbeck et al., 1969; Nakashima and Konigsberg, 1974).

To ascertain whether the A protein complex is present at one end of the virion, fl phages were incubated with the rabbit anti-fd A protein antibody and ferritin-conjugated goat anti-rabbit antibody and examined by electron microscopy (Figure 3). The ferritin-conjugated antibodies were all found localized at one end of the phage, as visualized on one or two phage particles (Figure 3A) or on rosette-like structures containing large numbers of phage joined by the antibody complex (Figure 3B,C). The number and size of complexes were greater in samples containing higher concentrations of A protein antibody but the same amount of phage and ferritin-conjugated goat anti-rabbit antibody. The phages contained in the rosette-like complexes were always connected to the ferritin–antibody complex at only one end of the virion, an event which would be expected if A protein is only present at one end. The results also suggested that the experiments were done in antigen (phage) excess. Technically, it was not possible to increase the ratio of anti-A protein antibody to phage to the point of antibody excess.

Control experiments showed that the large structure in the center of the rosette was probably a ferritin–antibody complex.

TABLE III: Ferritin-Antibody Complexes of fl Bacteriophage.[a]

Rabbit antibody	Ferritin-complex at one end of phage (%)				Ferritin along length[b]
	No complex on end	On end of singlets[b]	On end of doublets[c]	On end of aggregates[d]	
200 μg of anti-A protein	28.4	9.5	10.8	51.3	17.6
400 μg of anti-A protein	4.8.	1.1	0	94.1	
800 μg of anti-A protein	0	0	0	100	
400 μg of anti-sulfite oxidase	85	15	0	0	48.1
No antibody	79	21	0	0	21

[a] Fl phages were reacted with rabbit antibody and then with ferritin conjugated goat anti-rabbit antibody, negatively stained, and examined by electron microscopy as described under Experimental Procedures. Fifteen adjacent fields were examined. Approximately 150 phages were found in these fields for each sample and the percent of these phage in each structure (single, doublet, aggregate) was determined. [b] This refers to single ferritin particles at the end or along the length of phage as shown in Figure 6A,B. [c] This refers to two phage joined together via a ferritin-antibody complex as shown in Figure 5A. [d] This refers to three or more phages joined together in a rosette structure via a ferritin-antibody complex, as shown in Figures 5B,C.

When an equivalent amount of rabbit IgG against an unrelated antigen (rat liver sulfite oxidase) was substituted for the anti-A protein IgG, no rosettes or complexes of ferritin-antibody were observed (Figure 4A). Only a background of single ferritin particles was observed. If buffer was substituted for the anti-A protein IgG, essentially the same result was obtained (Figure 4B).

The number of phage present in the anti-A protein-ferritin complexes was quantitated as follows. A series of pictures from 15 adjacent fields was taken and analyzed for the number of phage complexed together at one end by the anti-A protein-ferritin complex (Figure 3). The number of single phage containing no ferritin or containing single ferritin particles at one end or along the length of the phage was also determined (Figure 4). This accounted for all of the 150 phage in the 15 fields. The results are shown in Table III. Only those experiments which had anti-A protein present as the rabbit antibody showed phage complexed together at one end. No such structures were observed if anti-sulfate oxidase IgG was used, or if rabbit IgG was omitted from the reaction. Therefore, fd anti-A protein will react with fl phage only at one end.

Discussion

In this paper, we have reported a procedure for isolating a complex containing the minor coat protein of bacteriophage fl using the detergent deoxycholate, which circumvented the problem of aggregation of the protein reported by others (Marco, 1975). The fl A protein complex prepared using deoxycholate has a molecular weight of 60 000, as determined by sodium dodecyl sulfate-urea-polyacrylamide gel electrophoresis. We have found that there are approximately three A protein complexes per phage, which appear to be localized at one end of the fl virion. The cross-reactivity of the anti-fd A protein antibody with our purified fl A protein complex and with fl phage indicates that the A proteins from fl and fd phages must have some similar structures in the phage particle. In addition, anti-fd A protein-antibody inactivates fl and fd phages at the same rate (data not shown).

It would appear that solubilization and gel filtration of the A protein in the presence of deoxycholate fail to resolve some protein component that *is* separated in the presence of sodium dodecyl sulfate and mercaptoethanol. We occasionally observe a very slight shoulder on the peak of purified fl A protein in sodium dodecyl sulfate gels which might indicate the presence of an additional large protein, but we have not been able to resolve it. Furthermore, when fl phage or purified A proteins radioactively labeled with an amino acid not found in the B protein are electrophoresed on gels, some radioactive material is observed migrating near the dye front. These additional protein moieties may represent biologically meaningful components of the A protein complex within the fl virion, which may participate in one or more of the several purported functions of the A protein in infection. This protein species is probably not the fl B protein, the bacterial pilus protein F pilin, bacterial flagellin, or one of the four major outer membrane proteins of *E. coli*. It might be the product of either fl genes 6 or 7, which is thought to be present on the fl virion in addition to the A and B proteins (Kornberg, 1974). Alternatively, the A protein may be synthesized in a precursor form, analogous to the B protein (Pieczenik et al., 1974; Konings et al., 1975). An amino- or carboxyl-terminal segment of the precursor could be cleaved from the mature A protein but remain in association with the A protein in the absence of sodium dodecyl sulfate and mercaptoethanol. We are attempting to purify this component of the complex from deoxycholate-purified A protein, subsequently fractionated in sodium dodecyl sulfate and β-mercaptoethanol containing solvents.

Purification using the detergent DOC may be preferable to sodium dodecyl sulfate procedures, because the isolated protein could be in a native and hence active conformation, as has been shown for other proteins in the presence of this detergent (Makino et al., 1973). Activity of DOC-purified A protein might be tested by determining whether such a complex has a higher efficiency of transfection of *E. coli* spheroplasts compared to protein-free fl DNA. This would be analogous to the experiments of Iglewski (1976) who showed that the RNA-assembly protein complex of R17 has a much higher specific infectivity, when assayed on spheroplasts or intact cells, than R17 RNA alone.

Kornberg (1974) has hypothesized that the adsorption protein may facilitate passage of the hydrophilic phage DNA through the hydrophobic milieu of the inner (cytoplasmic) membrane of the host cell by forming a membrane pore. This function implies that A protein might be amphipathic possessing a hydrophobic domain that interacts with the membrane and the hydrophobic region of the major coat protein, and one or more hydrophilic domains. If such a hydrophobic domain does exist in the A protein, it might act as a binding site for deoxycholate, as is the case with both cytochrome b_5 (Robinson and Tanford, 1975; Visser et al., 1975) and the major coat protein of fl (Makino et al., 1975; Woolford and Webster, 1975). By analogy, dissecting the A protein into

hydrophilic and hydrophobic domains with different functions might be possible with limited proteolysis. Perhaps the cleavage of the A protein to a smaller molecular weight form, upon binding and eclipse of the phage (Marco et al., 1975), represents proteolysis at a point between such domains, as occurs with cytochrome b in vitro (Robinson and Tanford, 1975). It is noteworthy that the putative pilot protein of R17, i.e., the assembly protein, is also cleaved upon binding of phage to cells (Krahn et al., 1972).

The availability of purified adsorption protein and of antibodies against this protein should greatly facilitate attempts to understand the diverse functions of this protein during phage infection and morphogenesis, and to understand its role in maintaining the structure of the phage.

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