INFECTION AND IMMUNITY, Feb. 1983, p. 939-947
0019-9567/83/020939-09$02.00/0

Vol. 39, No. 2

Isolation and Characterization of a Bacteriophage Factor That Confers Competence for Genetic Transformation to an Exfoliative Toxin-Producing Strain of *Staphylococcus aureus*

MATTHEW P. JACKSON,[1] JOHN DESENA,[1] JOHN LEDNICKY,[1] BARBARA MCPHERSON,[1] RICHARD HAILE,[1] ROBERT G. GARRISON,[2] AND MARVIN ROGOLSKY[1]*

Department of Biology and School of Medicine, University of Missouri, Kansas City, Missouri 64110,[1] and Veterans Administration Hospital, Kansas City, Missouri 64128[2]

Received 14 July 1982/Accepted 4 October 1982

Competence for genetic transformation in an exfoliative toxin-producing strain of *Staphylococcus aureus* was shown to be dependent on a virion factor that was isolated from a crude bacteriophage 80α lysate. This competence-conferring factor was completely separated from infectious virus particles after either centrifugation through a neutral sucrose velocity gradient or fractionation on a Sepharose 2B gel. Since the competence-conferring factor tends to aggregate, optimal separation was obtained after treatment of the phage factor with the detergent Nonidet P-40. The competence-conferring factor had a molecular weight between 3×10^6 and 20×10^6 and an approximate sedimentation coefficient of 252. The factor was neutralized after interaction with antiserum prepared against isolated infectious 80α virions. Electron microscopy of transforming cells that were exposed to isolated competence-conferring factor revealed a significant number of abnormally long and aggregated phage tail-like structures attached to the surface of recipient cells. This phenomenon was only observed in the presence of donor DNA, indicating that a phage tail-DNA-surface receptor complex might be one of the early steps in DNA-mediated transformation of *S. aureus*.

Bacteriophage in the presence of high concentrations of Ca^{2+} has been shown to be required for competence in DNA-mediated transformation of the staphylococcal phage group 3 strain 8325 (10, 12). Pattee (6) used transformation to construct an extensive genetic map on the strain 8325 chromosome. In past studies, our laboratory attempted to map a genetic determinant for exfoliative toxin A (ET A) synthesis on the staphylococcal chromosome (4, 8). Exfoliative toxin is responsible for a spectrum of clinical manifestations in humans which are collectively termed the scalded skin syndrome (7). Although numerous genetic markers were available for the phage group 3 8325 strain, we were unable to utilize them for genetic mapping studies, since DNA carrying the genetic determinants for ET A was from a phage group 2 strain, and intergroup transformation could not be accomplished. We therefore isolated our own phage group 2 markers and devised a transformation procedure that was dependent on mixing a crude lysate of the serological group B phage 80α with phage group 2 recipients (3, 4). After devising this procedure, we became concerned about how 80α conferred competence to our phage group 2 strains.

Studies by Sjöström and Philipson (10) indicate that competence in strain 8325 is dependent on an intracellular product from an early gene of phage φ11 in lysogenic recipients. However, Thompson and Pattee (12) made a number of observations which indicated that competence in strain 8325 was dependent on neither lysogeny nor an early phage gene product. Fourteen serological group B phage lysates were shown to confer competence to nonlysogenic strain 8325 recipients. Doses of UV irradiation which eradicated the plaque-forming activity of a phage lysate did not affect competence-conferring activity, and recovered recombinants were not lysogenic. These observations led to the suggestion that an external virion component mediated the transfer of DNA across the cell surface of a competent bacterium.

More recently, it was shown that centrifugation of 80α lysates through Ficoll gradients resulted in a partial separation of competence-conferring activity for strain 8325 and plaque-forming activity (1, 13). The competence-conferring activity could be inhibited by antiserum prepared against the isolated virions of a serological group B phage. Electron microscopy of Ficoll-enriched competence-conferring

factor (CCF) revealed an abundance of free phage tail structures (1). Phage 80α lysates and its associated CCF had considerable lytic activity against strain 8325 (1, 13) but not against the phage group 2, ET A-producing strain UT0002-19, which was used by our laboratory to assay for competence-conferring activity. The major objectives of the present studies were to find new and better procedures for the isolation of CCF and to provide insight into the properties and mechanism of action of the phage 80α factor which confers competence to an ET A-producing phage group 2 staphylococcal strain.

MATERIALS AND METHODS

Bacterial strains and phage. UT0002 is a phage group 2 (55/71) staphylococcal strain that contains both a chromosomal genetic determinant for ET A synthesis and an extrachromosomal genetic determinant for exfoliative toxin B (ET B) synthesis (7, 9, 14). Strain UT0002-19 was derived after overnight growth of strain UT0002 at 44°C (9). The substrain was cured of the plasmid for ET B synthesis but remained toxinogenic, since it still retained the genes for ET A synthesis. It was therefore pathogenic in that it was able to cause an impetigo, called the scalded skin syndrome, in humans (7). Strain UT0002-19 *nov-9* is a novobiocin-resistant derivative of strain UT0002-19. It was obtained after transforming strain UT0002-19 with DNA from strain MR103 *nov-9* (4). Strain ISP8 is a derivative of the phage group 3 strain 8325 and lacks the temperate phage ϕ11, ϕ12, and ϕ13 (12). Phage 80α is a serological group B phage that was isolated as a mutant of the group 1 typing phage 80 (5). Both phage 80α and its propogating strain ISP8 were supplied by P. A. Pattee.

Culture media and chemicals. All cultures were routinely maintained and subcultured on heart infusion agar (HIA) (Difco Laboratories, Detroit, Mich.). Stock cultures were maintained at 4°C on HIA. Trypticase soy broth (TSB) (BBL Microbiology Systems, Cockeysville, Md.) was used for resuspending competent cells and for the propagation and storage of phage. Both HIA and TSB were supplemented with 20 μg of thymine per ml. The selective medium used both to subculture strain UT0002-19 *nov-9* and for transformation experiments was prepared by supplementing HIA with 0.2 ml of novobiocin (0.2 mg/ml) (Sigma Chemical Co., St. Louis, Mo.). The top agar which was used to assay for plaque-forming activity consisted of 3% TSB and 0.5% Bacto-Agar (Difco). The bottom agar contained 3% tryptic soy agar (Difco) supplemented with 10 mM $CaCl_2$. Phage suspension medium (PSM) was the buffer used in dialyses and for storage of CCF. PSM consisted of 0.45 mM $CaCl_2$, 0.42 mM $MgSO_4$, 350 mM NaCl, and 30 mM Trizma base (Sigma) (pH 7.3).

Propagation of phage 80α. Phage 80α was propagated in strain ISP8 in TSB with 2 mM $CaCl_2$ according to the procedure of Martin and Rogolsky (3). This method yielded a crude lysate with a titer of approximately 10^{10} PFU/ml which was stable for several months.

Transformation. DNA for transformation was prepared from strain UT0002-19 *nov-9* according to the procedure of Martin and Rogolsky (3). Donor DNA carrying the *nov-9* marker was then transformed into a novobiocin-sensitive recipient UT0002-19 strain by the plate method, which has been previously described by this laboratory (3, 4). This procedure was modified in that either 1 ml of an 80α preparation or 0.1 ml of partially purified CCF was added to recipient cells. The plate method is much simpler than the conventional transformation procedure in that competent cells are taken directly from HIA plates after overnight growth at 37°C. Nov^r recombinants were selected on HIA containing 2 μg of novobiocin per ml. Transformation frequencies of approximately 10^{-6} were routinely obtained and were in the same range as those observed with the conventional transformation procedures (3, 4).

Separation of CCF from 80α virions by neutral sucrose velocity gradient centrifugation. Phage 80α was concentrated by centrifugation of 25 ml of crude lysate in an SW41 rotor (Beckman Instruments, Inc., Irvine, Calif.) for 3 h at 208,000 × *g* at 5°C. The pelleted phage was resuspended in 2 ml of PSM. This concentration step yielded approximately 10^{10} PFU/ml. Ultrapure gradient grade sucrose (Schwartz/Mann, Orangeburg, N.Y.) was mixed to 10 and 25% in PSM and adjusted to pH 7.3. Linear gradients were formed by mixing 5.6 ml of the 10% and 5.9 ml of the 25% sucrose solutions. Gradients were established in cellulose nitrate ultracentrifuge tubes which had been rinsed with monodibasic phosphate buffer (pH 7.0) to prevent possible nonspecific attachment of virus and CCF to the tubes. A 1-ml amount of the concentrated 80α preparation was treated with 0.06% Nonidet P-40 (NP40) (Sigma), with shaking at 200 rpm for 10 min at 30°C. A 0.3-ml sample of the NP40-treated phage preparation was layered onto the sucrose gradient. Ultracentrifugation at 153,000 × *g* for 30 min at 5°C in a Beckman SW41 rotor provided adequate migration of CCF and whole virus into the gradient. Twenty-two 0.5-ml fractions were collected from the bottom of the gradient. All fractions were dialyzed against PSM at 4°C overnight to eliminate residual sucrose. Spectrophotometric analyses (Zeiss spectrophotometer) at 280 nm were performed on each fraction, which was then filter sterilized through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.) and stored at 4°C. One hundred percent of the competence-conferring activity and 90% of the plaque-forming and competence-conferring activities for each fraction were performed within 3 weeks.

A reference marker consisting of 2.5×10^4 cpm of [5,6-^{3}H] uridine (specific activity, 40 Ci/mmol)-labeled poliovirus was cosedimented through a neutral sucrose gradient with an NP40-treated 80α preparation to determine the sedimentation coefficient of 80α and CCF. The sucrose gradient centrifugation procedure is the same as that described above, with the exception that a 0.3-ml sample of the poliovirus and phage mixture was centrifuged at 135,000 × *g* for 40 min at 4°C. Fractions were taken and assayed as described above, with the exception that each fraction was also counted in a Beckman model LS 3150T scintillation counter to determine the position of the poliovirus marker on the gradient. Poliovirus has a sedimentation coefficient (*s*) value of 160.

Separation of CCF from 80α virions by Sepharose 2B column chromatography. Phage 80α was precipitated from 1,200 ml of a crude lysate by slowly adding

small amounts of a saturated $(NH_4)_2SO_4$ solution (pH 7.3) with gentle stirring until an 80% (vol/vol) concentration was attained. After overnight incubation at 4°C, the preparation was centrifuged at 16,300 × *g* for 15 min at 4°C to collect the precipitate. The precipitate was dissolved in 120 ml of PSM and dialyzed against PSM at 4°C to remove residual $(NH_4)_2SO_4$. The concentrated phage preparation was centrifuged at 50,000 × *g* for 2 h at 4°C to partially separate phage from CCF. The pellet with the phage was discarded, and the supernatant was centrifuged at 210,000 × *g* at 4°C for 5 h to pellet CCF. The pellet was suspended in 12 ml of PSM. This final preparation or starting material had to be diluted 1/10 before application to a Sepharose 2B column so that the column would not be overloaded and a distinct separation of CCF and infectious virus could be obtained. A sample of the diluted starting material was then treated with 0.06% NP40 with shaking at 150 rpm at 23°C for 10 min. The treated starting material was then dialyzed overnight to remove residual detergent, and 1 ml of the dialysate was carefully layered onto a Sepharose 2B column.

Sepharose 2B columns (1.5 × 20 cm) were equilibrated with PSM. CCF samples were eluted with PSM at an operating pressure of 20 cm and a flow rate of 0.3 ml/min. The void volume (Vo) was determined to be 10 ml by eluting Blue Dextran 2000 under similar conditions. The total bed volume (Vt) in the column was 21.8 ml.

Preparation of antiserum against phage 80α. Phage 80α was isolated after Sepharose 2B gel chromatography as described above. Approximately 2.5×10^9 PFU were emulsified with an equal volume of Freund complete adjuvant (Difco) for the initial injection. Material for subsequent injections was similarly prepared, with the exception that the virus was emulsified with an equal volume of Freund incomplete adjuvant (Difco). Five separate 1-ml injections were given intramuscularly to two rabbits at 4-day intervals. The animals were exsanguinated 7 days after the final injection, and the resulting serum was stored at −55°C.

Electron microscopy. During transformation (3, 4), samples of strain UT0002-19 recipients were taken at various intervals after exposure to donor DNA and prepared for electron microscopy. Control cells, which were treated exactly like transforming cells except that they did not receive any donor DNA, were also prepared for electron microscopy. Both populations of cells were incubated with a CCF preparation that was completely separated from infectious virus after gel filtration through Sepharose 2B. This preparation contained 10^4 competence-conferring units (CCU) per ml and no PFU. Cells were fixed in equal parts of 6% glutaraldehyde and 0.2 M *S*-collidine buffer (pH 6.81) at 4°C for 1 and 15 h, respectively. The cells were then postfixed overnight at 4°C with 1% osmium tetroxide in a 0.1 M *S*-collidine buffer (pH 7.4) and then prestained with 3% uranyl magnesium acetate for 1 h. The stained cells were embedded in 2% agar, which was then cut into small cubes and dehydrated in a graded series of ethanol preparations. Embedding was accomplished by the method of Spurr (11). Thin sections were cut with a diamond knife on an LKB Ultrotome III and transferred to 300-mesh copper grids. The material on the grids was first stained with Reynold lead citrate for 5 min and then with 3% uranyl acetate in absolute ethanol for 10 min. Specimens were examined with a Hitachi electron microscope (model HU-11B-1) at 75 kV.

RESULTS

DNA-mediated transformation. In the transformation experiments, donor DNA carrying the *nov-9* marker was extracted from a phage group 2 strain UT0002-19 and transfered into novobiocin-sensitive strain UT0002-19 recipients. In past studies in our laboratory, the highest transformation frequencies were obtained with the *nov-9* marker (3, 4, 8). Transformation frequency is defined as the number of recombinants per number of total CFU. However, as observed by Thompson and Pattee (13) for phage group 3 strains, the same preparation of phage group 2 recipient cells exposed to the same 80α preparation under exactly the same conditions would result in diverse transformation frequencies. However, transformation values were found to be more consistent when they were expressed as CCU per milliliter, which is defined as the number of novobiocin-resistant transformants per milliliter of an 80α preparation. The phage group 2 UT0002-19 recipient, unlike the phage group 3 strain 8325 recipient (1, 13), is resistant to lytic infection by phage 80α. The lysis of strain 8325 by enriched CCF preparations with low PFU (1) was not observed with strain UT0002-19.

Separation of competence-conferring activity from plaque-forming activity by isopycnic CsCl gradient centrifugation. Since the densities of 80α phage and CCF were reported to be about 1.5 and 1.3 g/cm^3, respectively (13), a 0.5-ml preparation of 80α phage was mixed with distilled water and CsCl to attain a density of 1.5 g/cm^3. The mixture (11.5 ml) was then centrifuged to equilibrium at 130,000 × *g* for 30 h at 20°C. Fractions (0.5 ml) were then removed from the bottom of the tube and assayed for plaque-forming activity on strain ISP8 and for competence-conferring activity on strain UT0002-19. Extreme aggregation of both phage and CCF was observed, and no separation of these factors could be achieved. As observed by Thompson and Pattee (13), most of the virus pelleted with the remainder spread throughout the entire gradient. Multiple broad bands of CCF were seen over the lower ¾ of the gradient and in the pellet. Unusually low competence-conferring activity was recovered from the gradient. It was therefore concluded that isopycnic CsCl gradient centrifugation is not a satisfactory procedure to separate CCF and infectious 80α virions.

Separation of competence-conferring activity and plaque-forming activity by neutral sucrose velocity gradient centrifugation. Concentrated phage 80α lysates were centrifuged through neu-

tral sucrose gradients to determine whether competence-conferring activity could be separated from plaque-forming activity by this method and to determine the sedimentation coefficients of CCF and intact virus. Initially, we were able to separate the two activities on the gradient, but aggregation caused CCF to position itself into two or three bands. After examination of different concentrations of a number of detergents, it was found that treatment of an 80α lysate with a 0.06% solution of NP40 for 10 min at 30°C could disaggregate CCF without affecting competence-conferring activity. Therefore, the concentrated 80α lysate was treated with NP40, according to the conditions described above, before it was layered onto the sucrose gradient. The treated preparation contained 5×10^3 CCU/ml and 8×10^9 PFU/ml. The results of this procedure are depicted in Fig. 1. A distinct separation of competence-conferring activity and plaque-forming activity was achieved. Fraction 16 contained the peak of plaque-forming activity (2×10^7 PFU/ml) and no competence-conferring activity. The peak of competence-conferring activity was centered in fraction 19, which contained 1.7×10^3 CCU/ml and no plaque-forming activity. Optimal separation of the two factors was dependent on pretreatment with NP40. Neither the level of competence-conferring activity nor the level of plaque-forming activity was increased over that seen in the crude 80α lysate after separation of the factor for these activities on a sucrose gradient. Thus, separation of the two factors from each other does not enrich for the biological activity of either factor. The sedimentation coefficient of phage 80α was determined to be 387 after cosedimenting the phage with [5,6-^{3}H]uridine-labeled poliovirus (sedimentation coefficient, 160) in a neutral sucrose velocity gradient. After deter-

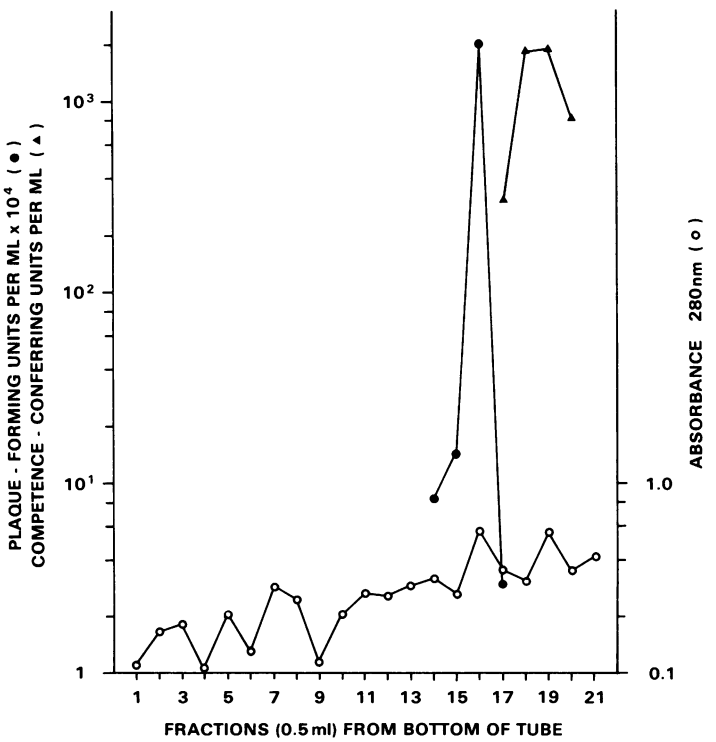

FIG. 1. Separation of competence-conferring activity from plaque-forming activity after centrifugation of a phage 80α preparation through a 10 to 25% neutral sucrose velocity gradient. Each fraction was assayed for PFU (●), absorbance at 280 nm (○), and competence-conferring activity (▲) on strain UT0002-19. One CCU is defined as one novobiocin-resistant recombinant per ml of a CCF preparation that was assayed. Our assay system for plaque-forming activity cannot detect less than 10 PFU/ml. Therefore, fractions not showing any PFU might contain as many as 10 PFU/ml. Our assay system for competence-conferring activity is sensitive enough to detect 10 recombinants per ml of a sample assayed for CCF. If more recipient cells were used for these transformation experiments or if experimental conditions were modified to enhance transformation frequencies, fractions not showing any competence-conferring activity might actually have some low competence-conferring activity.

mining the *s* value of phage 80α, it was then possible to use Fig. 1 to determine the *s* value of CCF, which was calculated to be 252.

Separation of competence-conferring activity and plaque-forming activity by Sepharose 2B gel chromatography. Sepharose 2B gel chromatography was examined for its ability to partially purify CCF. A crude lysate of 80α phage was concentrated after $(NH_4)_2SO_4$ precipitation. The concentrated suspension was then subjected to differential centrifugation for the purpose of partially separating intact virus from CCF, and the pelleted virus was discarded. The passage of this starting material, which was not treated with NP40, through a column of Sepharose 2B resulted in complete separation of plaque-forming activity, which was found in the void volume, and competence-conferring activity, which was retained by the column. However, three separate peaks of competence-conferring activity were observed. These three peaks were reduced to one if the starting material was pretreated with NP40 before gel filtration through Sepharose 2B (Fig. 2). The peak of plaque-forming activity (fraction 10) was eluted with the void volume. None of the fractions (8 to 13) that contained plaque-forming activity had competence-conferring activity, and none of the fractions (17 to 20) that contained competence-conferring activity had plaque-forming activity. One hundred percent of the competence-conferring activity that was added to the column was recovered. The fact that CCF but not intact virus was retained by the Sepharose 2B column indicates that the virus has a molecular weight of greater than 40×10^6 and CCF has a molecular weight of less than 40×10^6. Figure 3 also shows that poliovirus (approximate molecular weight of 3×10^6), which was cochromatographed with the starting material, is lighter than CCF. In

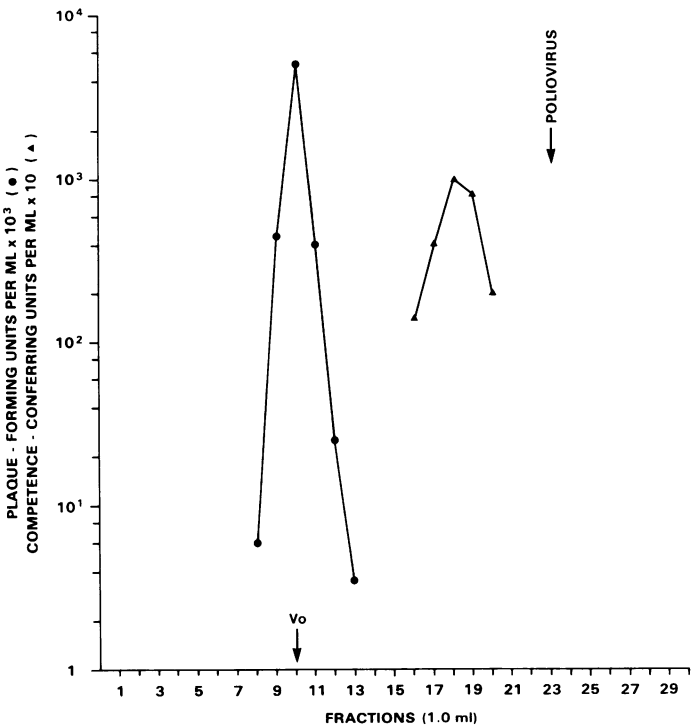

FIG. 2. Separation of competence-conferring activity from plaque-forming activity after fractionation of a phage 80α preparation by Sepharose 2B gel chromatography. Each fraction was assayed for PFU (●) and for competence-conferring activity (▲) on strain UT0002-19. One CCU is defined as one novobiocin-resistant transformant per ml of a CCF preparation that was assayed. The void volume (Vo) was 10 ml, and the total bed volume (Vt) was 21.8 ml. A [5,6-^{3}H]uridine-labeled poliovirus reference marker (approximate molecular weight, 3×10^6) was cochromatographed with the phage 80α preparation. Our assay system for plaque-forming activity cannot detect less than 10 PFU/ml. Therefore, fractions not showing any PFU might contain as many as 10 PFU/ml. Our assay system for competence-conferring activity is sensitive enough to detect 10 recombinants per ml of a sample assayed for CCF. If more recipient cells were used for these transformation experiments or if experimental conditions were modified to enhance transformation frequencies, fractions not showing any competence-conferring activity might actually have some low competence-conferring activity.

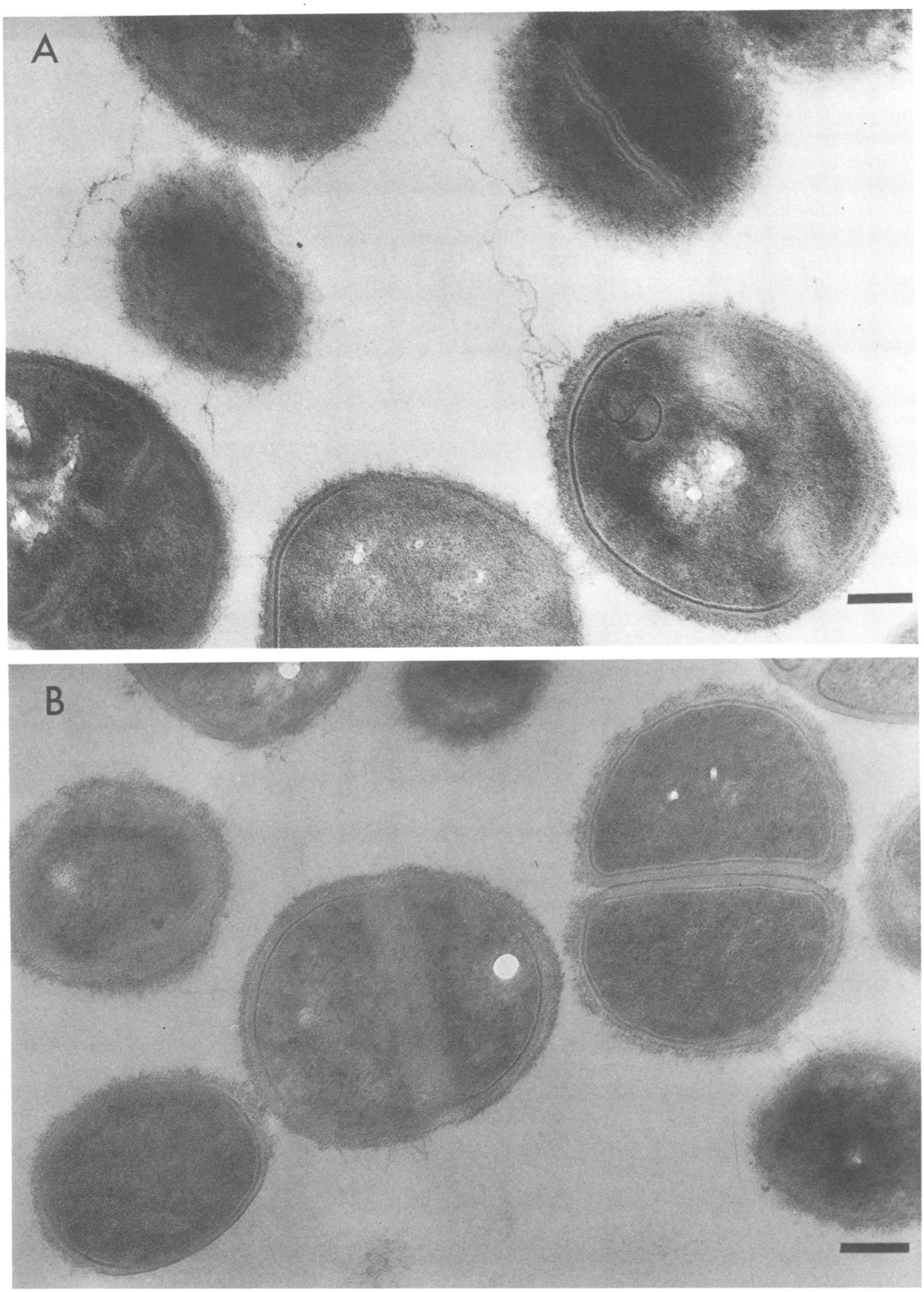

FIG. 3. Electron micrograph of transforming strain UT0002-19 cells. (A) Recipient cells in the process of transformation 8 min after the addition of donor DNA. The cells were exposed to CCF that was isolated after fractionation on a Sepharose 2B gel. (B) Recipient control cells were treated exactly like those in (A), except that they never received donor DNA. These cells were exposed to CCF but were never seen in association with the strands of fibrous structures seen in (A). Bars, 200 nm.

TABLE 1. Separation of competence-conferring activity and plaque-forming activity from a crude lysate of phage 80α

Prepn	PFU/ml	CCU/ml[a]	Transformation frequency[b]
Crude 80α lysate	1.5×10^9	7.1×10^4	1.4×10^{-6}
Concentrated 80α lysate[c]	5.2×10^9	7.5×10^4	7.7×10^{-7}
Centrifuged lysate[d]	4×10^8	6.7×10^4	8.3×10^{-7}
Starting material[e]	5×10^8	9.8×10^4	8.5×10^{-7}
Fraction 18 after Sepharose 2B gel chromatography (Fig. 1)	0	1×10^4	9.1×10^{-7}

[a] The number of strain UT0002-19 Nov^r recombinants per milliliter of a sample assayed for CCF.

[b] The number of strain UT0002-19 Nov^r recombinants per CFU in the assay mixture.

[c] Material precipitated from the crude lysate with 80% $(NH_4)_2SO_4$, centrifuged, and suspended into 1/10 of its original volume.

[d] Supernatant fluid after centrifugation of the concentrated lysate at 50,000 × *g* for 2 h at 4°C. The pellet from this differential centrifugation step contained 3×10^8 PFU/ml and less than 10 CCU/ml.

[e] Obtained after centrifugation of the concentrated lysate at 210,000 × *g* for 5 h at 4°C and suspending the pellet into 1/10 of its original volume.

another experiment, it was shown that CCF is retained by a Sepharose 4B gel. The molecular weight of CCF is therefore between 3×10^6 and 20×10^6. The biological activities of the preparations leading to the starting material that was used to obtain the data in Fig. 2 are shown in Table 1. After partial purification of CCF and complete separation of CCF from infectious virus by fractionation on Sepharose 2B gels, the competence-conferring activity was not enriched over that seen in the crude lysate (Table 1).

Inhibition of competence-conferring activity by antiserum prepared against phage 80α. Antiserum was prepared against intact phage 80α that was isolated after fractionation on Sepharose 2B gel columns. The *K* value of the antiserum was calculated to be 69 by the serum neutralization test of Eisenstark (2). A 10% (vol/vol) solution of this antiserum was interacted with 0.1 ml of a CCF fraction that was isolated after gel filtration through Sepharose 2B. This treatment resulted in a 99% reduction of the competence-conferring activity of the CCF fraction, indicating that CCF is of bacteriophage and not bacterial origin provided that 80α virions are not associated with any bacterial proteins.

Electron microscopy of strain UT0002-19 recipient cells undergoing transformation in the presence of partially purified CCF. Birmingham and Pattee (1) observed that an 80α preparation which was enriched for competence-conferring activity and isolated from a Ficoll gradient revealed large numbers of normal-sized free phage tails after electron microscopic examination. However, this CCF preparation still contained plaque-forming activity. The CCF preparation observed with an electron microscope in our laboratory was isolated after fractionation on a Sepharose 2B gel and contained no plaque-forming activity. It was thought that more could be learned about this CCF preparation if it were observed during transformation. Figure 3 reveals the results of such an observation. Figure 3A shows strain UT0002-19 Nov^s transforming cells that were interacted with the CCF preparation and incubated with donor DNA carrying the *nov-9* marker for 8 min. Figure 3B shows strain UT0002-19 Nov^s cells that were treated exactly like those shown in Fig. 3A, except that they received no donor DNA. The surfaces of transforming cells (Fig. 3A) are distinctly associated with long fibrous strands. These structures are not seen on cells that received no donor DNA (Fig. 3B). The fibrous strands observed in Fig. 3A appeared to be abnormally long and aggregated in comparison to that observed by Birmingham and Pattee (1). The possibility exists that the fibrous strands observed in Fig. 3A might represent aggregated phage tails complexed with DNA.

DISCUSSION

In 1974, Sjöström and Philipson (10) observed that DNA uptake in transformation of *Staphylococcus aureus* was dependent on a serological group B bacteriophage. However, the role of bacteriophage in the mechanism for competence has never been clearly defined. Sjöström and Philipson (10) attribute competence to the expression of an early phage gene, whereas Thompson and Pattee (12, 13) attribute competence to an extracellular phage component that functions at the cell surface. The results presented in this paper provide new data that support the latter hypothesis.

Pattee and co-workers (1, 13) showed that Ficoll gradients could be used to separate CCF and PFU, which indicated that plaque-forming activity and competence-conferring activity were controlled by separate entities. A complete separation of these factors on Ficoll gradients was not achieved, since fractions enriched for

competence-conferring activity still had plaque-forming activity. However, it has been shown here that both neutral sucrose velocity centrifugation and fractionation in Sepharose 2B gels completely separate the two activities. Optimal separation was obtained after disaggregation of CCF with NP40. Recovery of competence-conferring activity was much higher from the Sepharose gels than from the sucrose gradients. It has also been shown here that CCF and 80α virions differ in both their molecular weights and their sedimentation coefficients. The above results strongly indicate that phage 80α and CCF are separate and independent entities. Both the results reported here and those of Thompson and Pattee (13) have shown that antibody prepared against isolated serological group B phage neutralizes competence-conferring activity, which indicates that CCF is of bacteriophage origin. Additional evidence that CCF is an 80α virion component was derived from a sodium dodecyl sulfate-polyacrylamide gel analysis of purified 80α phage and Ficoll-enriched CCF that was passed through Matrex Gel Orange A (1). Three bands from each preparation were very similar. However, the CCF preparation lacked one phage band and contained two additional bands with vastly greater amounts of material than their corresponding phage bands.

In past studies from this laboratory (4), we calculated that the optimal multiplicity of infection of 80α in conferring competence to strain UT0002-19 was 4. It was therefore thought that four infectious phage virions were needed for each recipient cell to achieve maximal competence. However, it appears that this concept is now in error, since our results indicate that PFU are not associated with competence. Therefore, one should no longer measure competence of *S. aureus* in terms of multiplicity of infection.

The relationship of CCF to phage and the mechanism by which CCF interacts with the cell surface to induce competence remain unclear. Attempts to obtain CCF by disrupting phage virions were unsuccessful (13). This might indicate that CCF is a phage product which is synthesized during the virulent cycle but is not assembled into the complete virus. Electron microscopy was used by Birmingham and Pattee (1) and by our laboratory to gain insight into the identity of this phage by-product. Electron photomicrographs prepared by Birmingham and Pattee (1) of Ficoll-enriched CCF revealed an occasional intact but empty virion, large numbers of normal-sized phage tails, and aggregates that were believed to be phage adsorption organelles. No distinct morphological structure could be identified as CCF.

We thought that better insight into the identity of CCF could be obtained from electron photomicrographs of transforming cells that were exposed to CCF which was isolated from Sepharose 2B gels. One then might be able to observe selective interaction between CCF and recipient cells. This was thought to be the case in the interpretation of Fig. 3. The long fibrous strands associated with the surface of the recipient strain UT0002-19 cells could be aggregated phage tail structures that might be CCF. This interaction is only seen in the presence of donor DNA, which indicates that one of the steps in the transformation mechanism may involve the formation of a complex among DNA, CCF, and a cell surface receptor. The sedimentation value and molecular weight estimate of CCF indicate that it could be as much as six times heavier than a poliovirus particle, which falls into the range that one might expect for an 80α tail structure. Such a structure would not be expected to approach the size of the fibrous strands seen in Fig. 3A. However, it has been clearly shown that CCF readily aggregates, and such aggregates have the possibility of forming the fibrous strands seen in Fig. 3A. Finally, it is difficult to interpret why we observed that approximately 10% of the recipient cells bound the fibrous strands in the presence of donor DNA but only 1 in 10^6 cells per ml transformed. This might be explained by the fact that transformation is multifactorial and the formation of a CCF-cell surface receptor-DNA complex is only one of dozens of steps that occur during this process of genetic transfer in *S. aureus*.

ACKNOWLEDGMENT

This investigation was supported by Faculty Research grant K2-10440-5100 from the University of Missouri, Kansas City, to M.R.

LITERATURE CITED

1. **Birmingham, V. A., and P. A. Pattee.** 1981. Genetic transformation in *Staphylococcus aureus*: isolation and characterization of a competence-conferring factor from bacteriophage 80α lysates. J. Bacteriol. **148**:301–307.
2. **Eisenstark, A.** 1967. Bacteriophage techniques. Methods Virol. **1**:449–524.
3. **Martin, S. M., and M. Rogolsky.** 1980. DNA-mediated transformation in mutants of phage group 2 *Staphylococcus aureus*. Can. J. Microbiol. **26**:938–951.
4. **Martin, S. M., S. C. Shoham, M. Alsup, and M. Rogolsky.** 1980. Genetic mapping in phage group 2 *Staphylococcus aureus*. Infect. Immun. **27**:532–541.
5. **Novick, R. P.** 1963. Analysis by transduction of mutations affecting penicillinase formation in *Staphylococcus aureus*. J. Gen. Microbiol. **33**:121–136.
6. **Pattee, P. A.** 1980. Genetic markers of the *Staphylococcus aureus* chromosome, p. 120–123. *In* S. J. O'Brien (ed.), Genetic maps, vol. 1. National Cancer Institute, Bethesda, Md.
7. **Rogolsky, M.** 1979. Nonenteric toxins of *Staphylococcus aureus*. Microbiol. Rev. **43**:320–360.
8. **Rogolsky, M., S. M. Martin, S. C. Shoham, and B. B. Wiley.** 1981. Gene linkage groups in phage group 2 staphylococci, p. 529–534. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.

9. **Rogolsky, M., B. B. Wiley, and L. A. Glasgow.** 1976. Phage group II staphylococcal strains with chromosomal and extrachromosomal genes for exfoliative toxin synthesis. Infect. Immun. **13**:44–52.
10. **Sjöström, J. E., and L. Philipson.** 1974. Role of the ϕ11 phage genome in competence of *Staphylococcus aureus*. J. Bacteriol. **119**:19–32.
11. **Spurr, A. R.** 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**:31–43.
12. **Thompson, N. E., and P. A. Pattee.** 1977. Transformation in *Staphylococcus aureus*: role of bacteriophage and incidence of competence among strains. J. Bacteriol. **129**:778–788.
13. **Thompson, N. E., and P. A. Pattee.** 1981. Genetic transformation in *Staphylococcus aureus*: demonstration of a competence-conferring factor of bacteriophage origin in bacteriophage 80α lysates. J. Bacteriol. **148**:294–300.
14. **Wiley, B. B., and M. Rogolsky.** 1977. Molecular and serological differentiation of staphylococcal exfoliative toxin synthesized under chromosomal and plasmid control. Infect. Immun. **18**:487–494.