Temperate Bacteriophage Which Causes the Production of a New Major Outer Membrane Protein by *Escherichia coli*

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Under most conditions of growth, the most abundant protein in the outer membrane of most strains of *Escherichia coli* is a protein designated as "protein 1" or "matrix protein". In *E. coli* B, this protein has been shown to be a single polypeptide with a molecular mass of 36,500 and it may account for more than 50% of the total outer membrane protein. *E. coli* K-12 contains a very similar, although probably not identical, species of protein 1. Some pathogenic *E. coli* strains contain very little protein 1 and, in its place, make a protein designated as protein 2 which migrates faster on alkaline polyacrylamide gels containing sodium dodecyl sulfate and which gives a different spectrum of CNBr peptides. An *E. coli* K-12 strain which had been mated with a pathogenic strain was found to produce protein 2, and a temperate bacteriophage was isolated from this K-12 strain after induction with UV light. This phage, designated as PA-2, is similar in morphology and several other properties to phage lambda. When strains of *E. coli* K-12 are lysogenized by phage PA-2, they produce protein 2 and very little protein 1. Adsorption to lysogenic strains grown under conditions where they produce little protein 1 and primarily protein 2 is greatly reduced as compared to non-lysogenic strains which produce only protein 1. However, when cultures are grown under conditions of catabolite repression, protein 2 is reduced and protein 1 is increased, and lysogenic and non-lysogenic cultures grown under these conditions exhibit the same rate of adsorption. Phage PA-2 does not adsorb to *E. coli* B, which appears to have a slightly different protein 1 from K-12. These results suggest that protein 1 is the receptor for PA-2, and that protein 2 is made to reduce the superinfection of lysogens.

The outer membrane of *Escherichia coli* and many other gram-negative bacteria contains a group of major outer membrane proteins. These proteins have a molecular mass in the range of 35 to 45,000 and may make up as much as 70% of the total outer membrane proteins (16, 17). Analysis of these proteins in several laboratories indicates that there is not a single major protein but instead a mixture of several major polypeptides with similar molecular mass present in *E. coli*. The electrophoretic mobility of these polypeptides differ in different sodium dodecyl sulfate (SDS)-containing gel systems and depend upon the conditions of solubilization (2, 6, 8, 20). In addition, Schnaitman (22) has recently shown that these polypeptides are different in different strains of *E. coli*, and the amounts of the polypeptides vary with different growth conditions.

The most effective means of separating these major outer membrane proteins is to analyze proteins which have been boiled in SDS solu-

tion with the alkaline gel electrophoresis system described by Bragg and Hou (2), or with discontinuous buffer gel systems of the types described by Neville (11) or Laemmli (9). When outer membrane proteins from *E. coli* K-12 or *E. coli* B are examined with the Bragg-Hou gel system, two major protein bands are observed and these have been designated by Schnaitman (21) as proteins 1 and 3.

Protein 1 from *E. coli* BE has now been purified and characterized extensively (J. P. Rosenbusch, J. Biol. Chem., in press). This protein has a molecular mass of 36,500, and it binds to isolated peptidoglycan to form continuous sheets with regular hexagonal symmetry. This latter property has led Rosenbusch to term this the matrix protein of the outer membrane. One characteristic feature of this protein is that when it is solubilized in SDS solution without heating it migrates on gels as an aggregate with an apparent molecular mass much greater than its true molecular mass (20). Rosenbusch has

determined that protein 1 does not bind SDS until it is heated strongly, and this may be the reason for this property. This protein has been studied in other laboratories, and it is designated as protein I by Henning et al. (4), as protein A₁ by Bragg and Hou (2), and as protein B by Koplow and Goldfine (8). Under many conditions of growth, protein 1 is the most abundant protein (in terms of mass) in the *E. coli* outer membrane. Rosenbusch has shown that in *E. coli* B this protein is a single polypeptide.

Protein 3 is the major protein which has been termed "heat modifiable" by Bragg et al. (2, 14), because its response to heating in SDS solution is exactly the opposite of protein 1. When protein 3 is analyzed on SDS gels without heating, its apparent molecular mass is lower than that which is observed after the protein has been boiled in SDS. This protein is designated as protein II* by Henning et al. (4), and as a protein C by Koplow and Goldfine (8). Schnaitman (21) has shown that this protein is probably a mixture of two polypeptides, designated as proteins 3a and 3b.

Initial studies of outer membrane proteins in our laboratory were done with *E. coli* 0111_{B4} and its derivative strain J-5, and these strains have a very different outer membrane protein composition from *E. coli* K-12. The outer membrane of *E. coli* 0111_{B4} contains very little protein 1, and it contains another major outer membrane protein designated as protein 2 which migrates between proteins 1 and 3 on Bragg-Hou gels (21). Protein 2 has a different CNBr peptide profile than protein 1, suggesting that it is a different polypeptide. Protein 2 was missing from most common strains which were examined, but a protein with migration properties similar to protein 2 was present in three different isolates of *E. coli* 0111 and in an enteropathogenic strain of serotype 078 (22). In the latter strain, protein 2 completely replaced protein 1.

Because of the unusual distribution of these outer membrane proteins among various *E. coli* strains, we undertook an examination of other pathogenic *E. coli* strains, particularly those which produced enterotoxins. We have now examined 13 enterotoxin-producing strains, and eight of these produced a band with migration similar to protein 2. We also examined a series of enterotoxin-producing *E. coli* K-12 derivatives which were prepared by mating various enterotoxin plasmid-carrying strains with a single, well-marked K-12 strain. One of these K-12 strains was found to produce protein 2. From

this strain we were able to isolate a temperate phage which causes other K-12 strains to make protein 2 in place of protein 1. This report described the isolation of this phage, and its preliminary characterization.

MATERIALS AND METHODS

Bacteria and culture conditions. The bacterial strains used in this study are described in Table 1. For the gel analysis of outer membrane proteins cultures were grown on minimal medium (23) containing 10 µg of thiamine per ml and 50 µg of L-tryptophan per ml, with either 1% Casamino Acids or 0.5% glycerol as the carbon source. Other cultures were grown on L broth, which contained 10 g of tryptone (Difco) per liter, 5 g of yeast extract (Difco) per liter, and 5 g of NaCl per liter and which was adjusted to pH 7.0 before autoclaving. Glucose (5 g/liter) was added after autoclaving in some experiments, as noted in the text.

Isolation and analysis of outer membrane proteins. Cultures harvested at mid-log phase (5 × 10⁸ cells/ml) were broken with a French pressure cell, and the outer membrane was isolated by extracting the crude envelope with Triton X-100 as previously described (21). The Triton-insoluble outer membrane fraction (18) was then dissolved at 37°C in a solubilizing solution containing SDS, EDTA, and 2-mercaptoethanol (21). This either was applied directly to Maizel (pH 7.2) gels (21) or the sample was dialyzed overnight against an SDS-urea solution, boiled for 5 min, and analyzed with the Bragg-Hou (upper buffer pH 11.4) gel system (21). All gels contained 7.5% acrylamide except for those for analysis of CNBr peptides, which contained 12% acrylamide (22).

TABLE 1. *Bacterial strains*

Strain	Properties and source
<i>E. coli</i> CP95	Serotype O157:K ⁺ "V17". Wild type, <i>ent</i> ⁺ (ST only). Obtained from C. Gyles. Carries transmissible <i>ent</i> plasmid (3).
<i>E. coli</i> 711	K-12 derivative. <i>lac</i> ⁻ , <i>trp</i> ⁻ , <i>nal</i> ^r , <i>str</i> ^r , F ⁻ . Free of plasmid DNA (3). Obtained from S. Falkow by R. Guerrant.
<i>E. coli</i> 711(P95)	K-12 derivative. Transcipient of mating of CP95 with 711. Coded by Gyles as clone 711(95)4. Same properties as 711, plus <i>ent</i> ⁺ . Obtained from S. Falkow by R. Guerrant.
<i>E. coli</i> M107	K-12 derivative. <i>lac</i> ^{del} , <i>supD</i> , <i>strA</i> . Obtained from R. Kadner.
<i>E. coli</i> 1485F ⁺	Strain W1485 (1), wild-type K-12 cured of lambda. F ⁺ . Obtained from Coli Genetic Stock Center, Yale Univ.
<i>E. coli</i> 1485F ⁻	Obtained from W1485 by curing with acridine orange. F ⁻ .
<i>E. coli</i> B	Wild-type Hill strain. ATCC 23225.
<i>E. coli</i> J-5	Gal-epimeraseless derivative of <i>E. coli</i> 0111 _{B4} (19). Obtained from E. Heath.

Bacteriophage assays. Phage plaques were assayed on a basal agar consisting of L broth plus 1.6% agar and 4 ml of soft agar consisting of L broth plus 0.8% agar. Addition of either Ca^{2+} or Mg^{2+} to this medium reduced the plating efficiency of phage PA-2. Phage dilutions were prepared in L broth and preadsorbed prior to plating by mixing an equal volume of the phage dilutions and a seed culture consisting of a log-phase L broth culture of either *E. coli* 1485F⁺ or *E. coli* M107 containing 5×10^8 cells/ml. After 15 min at 37 C, 0.2 ml of this mixture was added to the soft agar at 50 C and plated. Except in this case of adsorption assays, plates were incubated at 37 C and plaques were counted after 8 to 12 h. Phage sensitivity was determined by cross-streaking log-phase bacterial cultures on basal agar plates as above, half of each plate having been swabbed with a phage lysate containing at least 10^6 PFU/ml. In the case of phage PA-2 these plates had to be scored after 4 to 6 h due to extensive growth of lysogens. Lysogenic strains were obtained by streaking out growth from the swabbed portion of these plates. Isolated colonies were restreaked and clones were tested for phage resistance and for production of phage by spotting chloroform-treated L broth cultures of the clones on seeded plates.

Adsorption of phage PA-2. Bacteria used for adsorption assays were grown for 3.5 generations in various test media to a culture density of 5×10^8 cells/ml. Concentrated phage lysates in L broth were added at a multiplicity of 0.1 PFU per cell and the mixture was incubated without shaking at 37 C. Samples were withdrawn at 1.5-min intervals and diluted 10^4 with L broth containing 200 μg of streptomycin per ml which had been prewarmed to 37 C. The dilutions were then allowed to stand for about 10 min, and then they were preadsorbed to a seed culture of *E. coli* M107 and plated as described above except that 200 μg of streptomycin per ml was added to the soft agar. Adsorption experiments were done using a temperature-sensitive mutant of phage PA-2 which gave clear plaques at 39 C but not at 30 C, and the plaque assay plates were incubated at 39 C. This was done to avoid the problem that the lysogenic cultures contained a few free phage. These free phage gave turbid plaques at 39 C and were readily identified. Control experiments showed that the rate of adsorption of wild-type phage and the clear plaque mutant to non-lysogenic cultures were the same, and also that streptomycin completely prevented phage replication in streptomycin-sensitive strains when added 12 min after infection.

RESULTS

Isolation of phages PA-1 and PA-2. *E. coli* 711(P95) is one of a number of *ent*⁺ strains of *E. coli* K-12 constructed by Gyles et al. (3). This particular strain was isolated after an overnight mating of the multiply marked K-12 derivative strain 711 with an *ent*⁺ porcine isolate designated as strain CP95. Analysis of the DNA of strain 711(P95) showed that it contained a

single, small plasmid DNA species (3).

We examined the outer membrane protein composition of strain 711(P95) using both the Bragg-Hou and Maizel gel systems (Fig. 1). When boiled samples of protein from strain 711 and strain 711(P95) were compared on Bragg-Hou gels, we found that protein 2 was present in strain 711(P95), whereas only proteins 1 and 3 were present in strain 711. When unheated protein was examined with the Maizel (pH 7.2) gel system, the characteristic broadening of the "A" band associated with the production of protein 2 (22) was observed in strain 711(P95) but not in strain 711. We also examined the outer membrane protein of the wild-type strain CP95 (Fig. 2) and found that this strain produced protein 2 and little protein 1.

These results suggested that strain 711 had acquired the ability to produce protein 2 during the mating with strain CP95. Since a protein with a migration similar to that of protein 2 is present in a number of wild-type strains of *E. coli* carrying *ent* plasmids, we felt that the production of protein 2 might be due to the plasmid present in strain 711(P95). This possibility was ruled out when this plasmid was transferred to other K-12 strains and these did not

FIG. 1. A comparison of the electrophoretic profiles of outer membrane protein from K-12 strain 711 and its *ent*⁺ derivative strain 711(P95). In the left hand panel the protein samples were prepared for electrophoresis by boiling in SDS-urea solution (21) and analyzed on Bragg-Hou gels. In the right hand panel the protein samples were dissolved in SDS solution without urea at 37 C and analyzed on Maizel gels. In this and all succeeding figures the top of the gel is to the left, and the gels were prepared, stained, and scanned as previously described (21). The numbers indicate proteins 1, 2, and 3, and the letters indicate the "A" and "C" peaks observed in unboiled samples (21).

FIG. 2. Bragg-Hou gel profile of outer membrane protein from strain CP95.

make protein 2. Strain 711(P95) was then examined to see if it contained a prophage which was not present in strain 711 and which might have been acquired during the mating with CP95.

A culture of strain 711(P95) was irradiated with UV light according to the conditions described by Miller (10) for lambda phage induction, and the resulting lysate was plated on *E. coli* 1485F⁺. Two distinct types of plaques were obtained. About 90% of the plaques were small (about 1 mm in diameter) with turbid centers, very similar to the plaques produced by lambda under the same plating conditions. The remainder of the plaques were slightly smaller and uniformly very turbid so that they could be seen only under optimum illumination. Single plaques of each type were selected, recloned several times, and used to prepare plate lysates (10). The phage which gave plaques with turbid centers was designated as PA-1 and the phage which gave smaller, uniformly very turbid plaques was designated as PA-2 (the designation PA referring to pathogen associated). Both phages were temperate, and lysogens were readily selected from a number of K-12 strains.

Both phages gave normal plaques and plating efficiency on K-12 strains lysogenic for phage lambda, and lysogens of both phages were sensitive to phages lambda, T4, T5, T6, T7, and BF23. Phage PA-2 also exhibits a normal plating efficiency on several lambda-resistant strains and the plating efficiency of PA-2 on wild-type K-12 strains is not affected by catabolite repression or induction of the maltose operon.

Effect of lysogeny on outer membrane proteins. When cultures of K-12 strains lysogenic for phage PA-1 were compared to non-

lysogenic cultures of the same strains, the outer membrane protein profiles were identical and neither strains produced protein 2. Apparently, phage PA-1 was present in strain 711 before mating with the various *ent*⁺ strains, since strain 711, strain 711(P95), and all of the other *ent*⁺ derivatives of strain 711 which we received from Falkow were resistant to phage PA-1 and produced PA-1 plaques when they were spotted on a sensitive strain. Phage PA-1 has not been studied further.

Strain 711(P95) was resistant to phage PA-2. However, all of the other *ent*⁺ derivatives of strain 711 which we received from Falkow and which did not produce protein 2 were sensitive to phage PA-2, as was strain 711. We have now tested more than 20 K-12 strains in our culture collection and all are sensitive to phage PA-2.

Figure 3 shows a comparison of the outer membrane protein from cultures of *E. coli* 1485F⁺ lysogenic and non-lysogenic for phage PA-2. The lysogenic culture produced protein 2 and little protein 1. Strains of 711 lysogenic for

FIG. 3. Bragg-Hou gel profiles of outer membrane protein from a non-lysogenic culture of K-12 strain 1485F⁺ and a culture of the same strain lysogenic for phage PA-2.

phage PA-2 have an outer membrane protein profile identical to that of 711(P95) as shown in Fig. 1. We have examined PA-2 lysogens of more than 10 different K-12 strains, and all have produced protein 2. Clearly, the production of protein 2 is a consequence of lysogeny and not the result of selection of bacterial mutants with altered outer membrane proteins. In addition to lysogens selected on plates as described, we have examined lysogens after single-cycle infection where the bacteria have had minimal exposure to superinfection. Lysogens selected in this fashion have also produced protein 2. Recently, we have been able to obtain hybrids of phage lambda and phage PA-2 (these will be described elsewhere) which have PA-2 immunity and plaque morphology but lambda host range. Lysogens of these hybrids, which use a completely different cell surface receptor, still produce protein 2.

When various pairs of lysogenic and non-lysogenic strains are compared, no outer membrane proteins other than proteins 1 and 2 appear to be different. As nearly as we can estimate from scans of stained gels and from the few labeled cultures we have examined, the amount of protein 1 in non-lysogens is the same as the sum of proteins 1 and 2 in lysogens grown under the same conditions. The relative amounts of proteins 1 and 2 produced by various lysogens grown under a particular condition is a characteristic of the strains from which the lysogens were prepared. The relative amounts of proteins 1 and 2 is determined by the level of catabolite repression (see Fig. 10 and reference 22) and this is known to vary among different *E. coli* strains, particularly those which have been heavily mutagenized. Strain 1485F⁺ is a wild-type K-12 strain and has not been mutagenized; this may be the reason lysogens of this strain resemble wild strains such as CP95 (Fig. 2) and H10407 (22) in having little protein 1.

Although phage PA-2 was isolated from a strain carrying a plasmid, there is no apparent effect of either the P95 plasmid or the F plasmid on either the growth of the phage or on the outer membrane protein profile. Lysogens of 1485F⁺ and 1485F⁻ have an identical outer membrane profile, as do lysogens of strains M107 and 711 with and without the P95 plasmid.

Protein 2 is identical in properties and in electrophoretic mobility when outer membrane proteins from the *E. coli* 0111 derivative strain J-5 and from strains of K-12 lysogenic for PA-2 are compared. To obtain further evidence for the identity of the protein from these two strains, we grew a culture of strain J-5 which

was labeled with [³H]leucine and a culture of strain 1485F⁺ lysogenic for PA-2 which was labeled with [¹⁴C]leucine. The cultures were grown with glycerol as the carbon source, since 1485F⁺ did not grow well on succinate. After harvesting, the cultures were mixed and the envelope was obtained. The "A" protein, which is a mixture of proteins 1 and 2, was isolated as previously described (21) by SDS-Sephadex chromatography before and after boiling in SDS solution. The resulting mixture of proteins from the two strains was cleaved with CNBr and analyzed on 12% polyacrylamide gels (22). The gel pattern is shown in Fig. 4. Peptides 3, 4, and 6 are characteristic of protein 1, and peptides 1, 2, and 5 are characteristic of protein 2. The peptide profiles from both strains appear to be identical. The difference in relative peak heights seen in Fig. 4 is due to the fact that strain J-5 makes considerably more protein 1 and less protein 2 than the lysogenic 1485F⁺ strain.

Growth and properties of phage PA-2.

Plate lysates of phage PA-2 yielded titers of no greater than 10⁸, and broth lysates were the most effective means of preparing high-titer phage stocks. Figure 5 shows a broth lysate which was sampled after lysis had reached a maximum, and again after the turbidity had increased. There is a rapid drop in phage titer after the point of maximum lysis. There was no lysis and few phage produced when cultures were grown in Fraser-Jarrel medium or in nutrient broth.

Routinely the lysates were harvested by adding a small amount of chloroform at the time when lysis reached a maximum. The cultures were shaken briefly and centrifuged at 7,000 × g

FIG. 4. Cyanogen bromide peptides from the "A" protein from a mixture of outer membrane proteins from strain J-5 (solid line) and from a culture of strain 1485F⁺ lysogenic for phage PA-2 (dashed line). The samples were run on a Maizel system gel containing 12% acrylamide, and the peptides are numbered as described previously (21, 22).

FIG. 5. Growth of a broth lysate of phage PA-2. The cultures are strain 1485F⁺ grown on L broth at 37 C. The dashed line shows an uninfected control culture, and the solid line shows a culture infected at 0 at a multiplicity of 0.1 PFU/cell. The numbers indicate the titer in PFU per milliliter at two points during the lysis and regrowth of the infected culture.

for 10 min. Such clarified lysates were only moderately stable when stored in the cold, and the titer generally dropped from 2- to 10-fold in 1 week. Neither Mg²⁺ nor EDTA stabilized the lysates. When such lysates were spun at higher speeds to sediment the phage (50,000 $\times$ g for 1 to 2 h) there was complete inactivation of the phage. Pellets after such centrifugation were examined by electron microscopy, and they were found to contain a mixture of cell debris and empty phage particles. Similar inactivation also occurs when phage lysates are concentrated by ultrafiltration. Phage PA-2 could be concentrated without inactivation by the addition of NaCl and polyethylene glycol 6000 (24), but this is not a good routine procedure since it is difficult to separate the phage from the polyethylene glycol precipitate without large losses of phage.

In a few experiments it was possible to obtain small amounts of phage free of polyethylene glycol which could be banded on CsCl gradients prepared in T2 buffer (5). Rough measurements indicated that the phage banded in CsCl gradi-

ents at a density of about 1.45. When phage banded on CsCl gradients were dialyzed into 2% phosphotungstic acid, pH 6.5, and examined in the electron microscope, about half of the phage particles appeared intact, with full heads. The morphology of the phage is shown in Fig. 6. The phage is identical to phage lambda (7) consisting of an isometric (probably icosahedral) head 0.06- μ m in diameter with easily seen capsomeres and a simple tail 0.15- μ m long. In some cases (not shown) a single tail fiber similar to that of lambda (7) could be seen.

Figure 7 shows a single-step growth curve of phage PA-2. Half of the phage added to the culture were adsorbed during the 5-min incubation prior to dilution, and as based on the number of adsorbed phage the burst size was about 200 particles. This represents a minimum burst size, since some cells were undoubtedly lysogenized. Phage PA-2 does not appear to cause lysis from without. In one experiment, a log-phase culture of strain M107 containing 2×10^8 cells per ml was infected at a multiplicity of 10^3 PFU per bacterium. This culture was incubated for 30 min at 37 C, and during this incubation there was no decrease in turbidity indicative of lysis.

Adsorption of phage PA-2 to *E. coli* B and to lysogenic and non-lysogenic *E. coli* K-12. *E. coli* B is resistant to phage PA-2 when tested by cross-streaking. Figure 8A shows that this is due to lack of adsorption of the phage to *E. coli* B. This is one indication that protein 1 may be the receptor for phage PA-2, since protein 1 from this *E. coli* B strain exhibits a different electrophoretic mobility in the Bragg-Hou gel system as compared to protein 1 from *E. coli* K-12 (Fig. 9).

Figure 8A also shows the adsorption of phage PA-2 to a non-lysogenic culture of strain 1485F⁺, and to a culture of the same strain lysogenic for phage PA-2. The rate of adsorption to the lysogen is much slower than the rate of adsorption to the non-lysogen. The cultures in Fig. 8A were grown on L broth, which produces a relatively low level of catabolite repression. Essentially the same result was obtained when the lysogenic and non-lysogenic strains were grown on minimal medium with Casamino Acids as the carbon source (Fig. 8B), a medium which results in even lower catabolite repression. Both of the Casamino Acids-grown cultures showed slightly reduced rates of adsorption as compared to the cultures grown on L broth. However, when the lysogenic and non-lysogenic cultures were grown on L broth plus glucose, a condition resulting in a high level of catabolite

FIG. 6. Electron micrograph of phage PA-2 after purification in a CsCl gradient. Phosphotungstic acid negative stain. The bar indicates 0.1 μ m.

FIG. 7. Single-step growth curve of phage PA-2. A log-phase culture of strain 1485F⁺ containing 5×10^8 cells/ml was infected with phage PA-2 at a concentration of 1.5×10^8 PFU/ml. The infected culture was allowed to stand for 5 min at 37 C, and then was diluted 10^4 into prewarmed L broth and incubated on a shaker at 37 C. At various times after dilution, samples were removed, shaken gently with chloroform, and titered. The arrow indicates the number of phage added to the culture.

FIG. 8. Adsorption of phage PA-2 to various bacteria. Adsorption was measured as described. The open triangles indicate adsorption to *E. coli* B, the open circles adsorption to strain 1485F⁺, and the closed circles adsorption to strain 1485F⁺ lysogenic for phage PA-2. (A) Cultures were grown in L broth; (B) growth was in minimal medium plus Casamino Acids; (C) growth was in L broth plus 0.5% glucose. In all cases the adsorption mixture contained 5×10^8 cells/ml and 5×10^7 phage/ml.

repression, the adsorption to both cultures was the same (Fig. 8C). Figure 10 shows the major outer membrane proteins of the lysogenic and non-lysogenic cultures grown under exactly the same conditions of growth as the cultures used in the experiments shown in Fig. 8A and C. The major outer membrane proteins of the non-

FIG. 9. Comparison of the major proteins of *E. coli* K-12 (strain M107) and *E. coli* B on Bragg-Hou gels. The samples were prepared in an identical fashion and the same amounts of protein were applied to the gels, which were run simultaneously in the same gel bath. The migration of protein 3 was identical for both strains, and the dashed lines have been added to facilitate comparison.

lysogenic culture were essentially the same when grown in L broth with or without glucose. The addition of glucose to the L broth caused an increase in protein 1 and a decrease in protein 2 in the case of the culture lysogenic for PA-2. This is similar to the effect of catabolite repression on the level of protein 2 in *E. coli* 0111 which was reported previously (22).

DISCUSSION

Many cases are known in which temperate phages confer upon lysogenic cells properties which have no apparent role in lysogeny, and these phenomena are termed lysogenic conversion. These phenomena include the production

of toxins which are released from the cell, and mechanisms to exclude superinfection by other (or the same) phage. An extensive series of temperate phages of *Salmonella* and *Shigella* cause alterations in the cell surface brought about by changes in the lipopolysaccharide O side chains due to glucosylation or acetylation of these carbohydrates, or to alterations in the specificity of sugar polymerases involved in carbohydrate synthesis (12). In the cases which have been studied, the portion of the O side chain which is altered also functions as the phage receptor, and thus the alteration serves to exclude superinfection.

This report describes the isolation of a temperate phage, PA-2, which can bring about a similar change in the major proteins of the cell surface. In non-lysogenic strains of *E. coli* the protein we term protein 1 accounts for as much as 50% of the outer membrane protein and is one of the most abundant proteins in the *E. coli* cell. The recent micrographs of Rosenbusch indicate that this protein covers much of the cell surface. In cultures lysogenic for phage PA-2, the amount of this protein is greatly reduced, and in

FIG. 10. Effect of catabolite repression on the major outer membrane proteins of strain 1485F⁺ and the same strain lysogenic for phage PA-2. The cultures were identical to the cultures used in Fig. 8A and C. Only the major proteins are shown as they appeared on Bragg-hou gels, and there were no other differences between the gel profiles of the various cultures.

its place a protein with a different CNBr peptide profile and a different migration on alkaline SDS gels is produced. Proteins 1 and 2 are very similar in molecular weight and in solubility properties, and recent studies (A. Summers and D. Diedrich, unpublished data) indicate that these proteins are similar in amino acid composition.

It appears that the protein alteration caused by phage PA-2 is analogous to the alteration of lipopolysaccharide by the *Salmonella* and *Shigella* phages. In this report we describe two lines of evidence which indicate that protein 1 may be the receptor for phage PA-2. First, in our strain of *E. coli* B protein 1 exhibits an altered electrophoretic mobility as compared to the same protein from sensitive K-12 strains, and this strain of *E. coli* B cannot bind phage. Second, lysogens which make protein 2 in place of protein 1 exhibit a reduced ability to bind phage PA-2. When we reverse the effect of lysogenic conversion by growing lysogenic cultures under conditions of high catabolite repression, we also enhance the ability of the lysogenic cells to bind phage so that they become more similar to non-lysogenic cells.

Very recent data (which will be described in detail elsewhere) provides additional evidence that protein 1 is the receptor for phage PA-2. P. Bassford in our laboratory has obtained a clear-plaque mutant of PA-2 which does not lysogenize. Using this phage mutant, he has selected and characterized a number of PA-2-resistant mutants from strain 1485F⁻. Of six PA-2-resistant mutants which have been examined in detail, four had greatly reduced levels of protein 1 in the outer membrane and two had no detectable protein 1 in the outer membrane. We have also examined the PA-2 sensitivity of two classes of colicin-tolerant mutants, *tol* 4 and *tol* 14, which are completely missing protein 1 (J. Davies and P. Reeves, submitted for publication). Both of these mutants were resistant to phage PA-2, whereas the K-12 parent of these mutants was sensitive to PA-2.

If protein 1 is the receptor for phage PA-2, this might explain the instability of phage lysates and the difficulties encountered in purification of this phage. Protein 1 is very abundant as compared to other protein phage receptors, such as those for lambda (13) and BF23 (15). Rosenbusch has estimated that in the case of *E. coli* B there are more than 10⁶ molecules of protein 1 (matrix protein) per cell.

We can speculate that lysogenic conversion which results in the reduction in amount of a phage receptor protein on the surface of the cell

and the replacement of this protein by a similar protein which is not a receptor for the phage might be of selective advantage to the phage. In an environment (such as in a phage plaque, or its equivalent in nature) containing a large number of phage particles, lysogenic cells might be protected from the damaging effects of multiple superinfection. Likewise, such a lysogenic conversion might aid in dissemination of phage particles to infect sensitive cells, since lysogenic cells would be less likely to bind free phage. We can also speculate that the change from protein 1 to protein 2 on the surface of the host cells might have important considerations in terms of bacterial ecology and pathogenesis. Our survey on the presence of a protein similar to protein 2 in enteropathogenic *E. coli* strains indicates that phages similar to PA-2 may be widely distributed in nature. It should be emphasized that the relationship of phage PA-2 or protein 2 to pathogenesis is entirely circumstantial, and the phage has in no way been implicated in any pathogenic process. On the contrary, we have been unable to find any relationship between this phage and either sex factors or other plasmids which determine pathogenicity.

Phage PA-2 is clearly distinct from phage lambda in plaque morphology, in immunity substance, and in its receptor on the cell surface. PA-2 will plaque normally on lambda lysogens or lambda-resistant strains, and lambda will plaque normally on PA-2 lysogens and on the recently isolated PA-2-resistant mutants. However, these phages are quite similar. They are identical in morphology and appear to use somewhat similar proteins on the cell surface as receptors. Very recently, we have found that hybrids of lambda and PA-2 are readily obtained after simultaneous infection with both phage. Thus, PA-2 appears to be a member of the "lambdoid" group of phages. Further characterization of PA-2 must await purification of the phage and characterization of its nucleic acid.

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LITERATURE CITED

1. Bachman, B. J. 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. *Bacteriol. Rev.* 36:525-557.
2. Bragg, P. D., and C. Hou. 1972. Organization of proteins

- in the native and reformed outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **274**:478-488.
3. Gyles, C., M. So, and S. Falkow. 1974. The enterotoxin plasmids of *Escherichia coli*. J. Infect. Dis. **130**:40-49.
  4. Henning, U., F. Hohn, and I. Sonntag. 1973. Cell envelope and shape of *Escherichia coli* K-12. Eur. J. Biochem. **39**:27-36.
  5. Hershey, A. D., and M. C. Chase. 1952. Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. **36**:39-56.
  6. Inouye, M., and M. Yee. 1973. Homogeneity of envelope proteins of *Escherichia coli* separated by gel electrophoresis in sodium dodecyl sulfate. J. Bacteriol. **113**:304-312.
  7. Kellenberger, E., and R. S. Edgar. 1971. Structure and assembly of phage particles, p. 271-295. In A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, New York.
  8. Koplow, J., and H. Goldfine. 1974. Alterations in the outer membrane of the cell envelope of heptose-deficient mutants of *Escherichia coli*. J. Bacteriol. **117**:527-543.
  9. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680-685.
  10. Miller, J. 1972. Experiments in molecular genetics, p. 45. Cold Spring Harbor Laboratory, New York.
  11. Neville, D. M., Jr. 1971. Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. J. Biol. Chem. **246**:6328-6334.
  12. Nikaido, H. 1973. Biosynthesis of lipopolysaccharide, p. 131-208. In L. Leive (ed.), Bacterial membranes and walls. Marcel Dekker, Inc., New York.
  13. Randall-Hazelbauer, L., and M. Schwartz. 1973. Isolation of the bacteriophage lambda receptor from *Escherichia coli*. J. Bacteriol. **116**:1436-1446.
  14. Reithmeier, R. A. F., and P. D. Bragg. 1974. Purification and characterization of a heat-modifiable protein from the outer membrane of *Escherichia coli*. FEBS Lett. **41**:195-198.
  15. Sabet, S. F., and C. Schnaitman. 1973. Purification and properties of the colicin E3 receptor of *Escherichia coli*. J. Biol. Chem. **248**:1796-1806.
  16. Schnaitman, C. 1970. Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli*. J. Bacteriol. **104**:890-901.
  17. Schnaitman, C. 1970. Comparison of the envelope protein compositions of several gram-negative bacteria. J. Bacteriol. **104**:1404-1405.
  18. Schnaitman, C. 1971. Solubilization of the cytoplasmic membrane of *Escherichia coli* by Triton X-100. J. Bacteriol. **108**:545-552.
  19. Schnaitman, C. 1971. Effect of ethylenediaminetetraacetic acid, Triton X-100, and lysozyme on the morphology and chemical composition of isolated cell walls of *Escherichia coli*. J. Bacteriol. **108**:553-563.
  20. Schnaitman, C. 1973. Outer membrane proteins of *Escherichia coli*. I. Effect of preparative conditions on the migration of proteins in polyacrylamide gels. Arch. Biochem. Biophys. **157**:541-552.
  21. Schnaitman, C. 1974. Outer membrane proteins of *Escherichia coli*. III. Evidence that the major protein of *Escherichia coli* 0111 outer membrane consists of four distinct polypeptide species. J. Bacteriol. **118**:442-453.
  22. Schnaitman, C. 1974. Outer membrane proteins of *Escherichia coli*. IV. Differences in outer membrane proteins due to strain and cultural differences. J. Bacteriol. **118**:454-464.
  23. White, D. A., W. J. Lennarz, and C. Schnaitman. 1972. Distribution of lipids in the wall and cytoplasmic membrane subfractions of the cell envelope of *Escherichia coli*. J. Bacteriol. **109**:686-690.
  24. Yamamoto, K. R., B. M. Alberts, R. Benzinger, L. Lawhorne, and G. Treiber. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large scale virus purification. Virology **40**:734-744.