Alteration of the *Escherichia coli* Membrane by Addition of Bacteriophage T4 Proteins Synthesized After Infection

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Many T4-induced proteins were found associated with the *Escherichia coli* membrane during infection. Some of these were apparently ionically bound, but many could be identified as integral parts of the inner and outer bacterial membranes by their selective solubilities in guanidine or Sarkosyl. The synthesis and insertion of these proteins into the bacterial membrane were temporally controlled and, once in the membrane, these proteins were stably integrated. Host membrane protein synthesis continued after infection of non-UV-irradiated cells, but was not present if the cells were irradiated. There was no major redistribution or loss of bacterial proteins from *E. coli* membranes as a consequence of T4 infection.

The growth of phage T4 apparently involves an interaction of the phage components with the bacterial membrane. (i) It is known that the infecting and replicating T4 DNA molecules are bound to the membrane for some time during the eclipse period and that the DNA is detached during phage maturation (7). (ii) The *Escherichia coli* membrane is modified during infection because the cells become resistant to lysis from without (31), can be lysis inhibited (4), exclude superinfecting T4 (6) and ghosts (5), and are altered in their permeability (23). Some of these functions are known to be phage coded and require protein synthesis after infection for their elicitation (8, 14, 23, 29). (iii) The bacterial membrane has been proposed as the site of assembly of T4 capsids (25) and baseplates (24).

Because of the interest in the host membrane as a dynamic structure involved in T4 synthesis, we initiated a study several years ago to identify the proteins associated with the membrane after infection. Previously, we showed that the rIIA (9) and rIIB (21, 32) proteins were membrane bound and, subsequently, other T4 membrane-associated proteins were identified (1, 13, 16, 22, 26). In the present investigation, we further catalog the appearance on the *E. coli* membrane of T4-coded proteins synthesized after infection. The time of addition of these proteins to the membrane and the effect of T4 infection on bacterial membrane proteins are also described.

MATERIALS AND METHODS

**Bacteria and phage.** *E. coli* B and strain H-150, which is a mutant of *E. coli* B that requires high levels of K+ in the medium for growth, were used. The same results were obtained with either strain. Wild-type phage T4 and amB22 (a phage mutant in gene 43) were used in most of the experiments. Many other amber and deletion mutants in a number of other genes were used for identifying gene products (19, 30). All experiments and growth of cells were done at 37°C.

**Media and growth conditions.** Cells were cultured in M9 medium (1 g of NaCl, 0.13 g of MgSO4, 3 g of KH2PO4, 6 g of Na2HPO4, and water to 1 liter) containing 0.4% glucose. Where indicated, cells were irradiated with UV at 5,850 ergs/mm² to suppress host protein synthesis. The cells were irradiated at 280 nm with shaking at 50 rpm in M9 medium in dishes prepared so that the depth of the cell suspension was 2 mm. All protein synthesis after infection of irradiated cells is phage specific. Growth of the bacteria and infection by phage have been described (2). The cultures were labeled with a 14C-amino acid mixture (Amersham/Searle) as indicated in the legend to each figure. In all experiments, there was sufficient amino acid to ensure linear incorporation for the duration of the experiment.

**Isolation of membranes.** Membranes and the soluble proteins of the cell were isolated as previously described (10, 17). It was shown in control experiments that, in T4-infected cells, the method was appropriate to cleanly separate membranes from the soluble components of the cell. The long centrifugation sediments even the smallest membrane fragments.

**Analysis of proteins by dodecyl sulfate-polyacrylamide gel electrophoresis.** The preparation of slab gels and electrophoresis were done according to the procedures described previously (18, 27). Samples were subjected to electrophoresis on 7.5, 10, or 12.5% gels depending on the experiment and the requirements for separation of the proteins. Samples (approximately 50,000 cpm) were dissolved in 0.0625 M Tris-hydrochloride, pH 6.8, 5% 2-mercaptoethanol,
20% glycerol, and 1 to 2% sodium dodecyl sulfate and heated for 2 min at 100°C. Electrophoresis was at 30 mA per slab for about 3.5 h. After electrophoresis, the slabs were immediately dried (11) without fixation or staining, and the dried gels were exposed to Kodak NS2T no-screen X-ray film for 1 to 2 weeks.

RESULTS

Membrane-associated proteins synthesized after infection of E. coli with phage T4. The phage-induced proteins that are associated with the bacterial membrane after 15 min of infection were displayed by polyacrylamide gel electrophoresis. This was done by using wild-type T4 and T4 amB22 (a gene 43 mutant, lacking DNA polymerase). The use of the gene 43 mutant ensures that no mature phage are made during the infection and that what is observed is not merely a contamination of the membrane fraction with mature phage during sedimentation. Since these experiments were done with UV-treated cells, all the proteins are phage specific.

As can be seen in Fig. 1, many proteins syn-

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**Fig. 1.** Dodecyl sulfate-polyacrylamide gel electrophoresis of extracts of phage-infected E. coli. Two hundred-milliliter cultures of E. coli H-150 were grown in M9 medium to 5 × 10⁶ cells per ml. The cells were exposed to UV irradiation at 5,850 ergs/mm² and then shaken for 30 min at 37°C. The cells were then infected at a multiplicity of 5 phage per cell, and 0.5 μCi of 14C-labeled amino acid mixture per ml (final concentration; Amersham/Searle) was added. Fifteen minutes after infection non-radioactive Casamino Acids mixture (1 mg/ml) was added. The cells were incubated for an additional 5 min, and then the cells were extracted as outlined in Materials and Methods. (A) T4 amB22 (amber mutant in gene 43); (B) T4 wild-type-infected cells. Samples of cells (C), membranes (M), and soluble proteins (S) were analyzed on 7.5 and 12.5% gels. Some gene products are given by the gene number when known. Molecular weights were determined by comparison with calibration curves obtained by running protein standards on similar gels. Individual proteins were identified in gels by comparison with amber and deletion mutants in known genes and by comparison with their known molecular weights (19, 30).
thesized during the first 15 min after infection are associated with the bacterial membrane fraction. By comparison with Fig. 6, it can also be seen that the proteins isolated from uninfected cell membranes are completely different from those integrated into infected cell membranes. Approximately 35% of the total radioactivity incorporated into protein 0 to 15 min after infection (either the amB22 or wild type) sedimented with the membranes (average of several separate experiments). Others (28) found up to 70% of the proteins to be membrane associated, but these were early proteins made 4 to 7 min postinfection. It can also be seen that many of the proteins are found either exclusively in the membrane fraction or in the supernatant fraction of the infected cell. The majority of the proteins are distributed between the soluble and membrane fractions of the cell and may represent soluble proteins that associate artifactually with the membranes. More proteins are observed in cells infected with T4 wild type than with amB22. This is because late proteins are not made in the amB22-infected cells. Because of the large number of proteins in membranes from wild-type-infected cells, it is difficult to accurately identify specific gene products.

It was also observed that membrane proteins made early in infection are still associated with the membranes later in infection. In this experiment, cells were labeled with 14C-amino acids 0 to 5 min after infection, a large excess of nonradioactive amino acids was then added, and the infection was continued for another 10 min. As can be seen in Fig. 2, the same proteins found in the membrane 5 min after infection are present after the chase. Therefore, all of these proteins are stably associated with the membranes during early infection.

Extraction of membranes with guanidine-hydrochloride has been shown to have no effect on
protein-lipid interactions (26). Consequently, this agent can be used to remove proteins that may be electrostatically bound to the membranes. Membranes isolated from T4-infected cells were treated with 4 M guanidine-hydrochloride. The treated membranes and the proteins removed by this procedure were examined by polyacrylamide gel electrophoresis. Approximately 50% of the radioactivity is removed from membranes by the guanidine-hydrochloride. As is shown in Fig. 3, this treatment removes many proteins, but many others are not affected by this treatment.

Extraction of membranes with Sarkosyl selectively extracts membrane proteins without affecting those of the cell wall (1, 12, 13). Consequently, this agent can be used to discriminate between cytoplasmic membrane (Sarkosyl-soluble) and cell wall (Sarkosyl-insoluble) proteins. Membranes isolated from T4-infected cells were treated with Sarkosyl, and the soluble and insoluble proteins were analyzed by gel electrophoresis. In agreement with previous work (16, 27), approximately 72% of the radioactivity was solubilized by the detergent. It can be seen in Fig. 3 that Sarkosyl removes more proteins than guanidine (especially several high-molecular-weight species).

It is important to remember that, in these analyses, the same amount of radioactivity is analyzed for each sample (approximately 50,000 cpm). Therefore, the intensity of the bands corresponding to proteins that are not extracted is increased in the Sarkosyl-treated membranes. We assume that these bands identify the proteins that are associated with the cell wall.

Kinetics of synthesis of phage membrane proteins. The kinetics of synthesis of the membrane-associated proteins is given in Fig. 4. In this experiment, infected cells were labeled for short intervals of time during infection by wild-type T4. The cells were fractionated, and the membranes were isolated and subjected to polyacrylamide gel electrophoresis. The kinetics of the appearance of specific proteins on the membrane and the temporal relationship among the different proteins can be seen.

Host membrane proteins are made after infection. In most of the experiments described in this investigation, the cells were exposed to UV irradiation before infection. This treatment and the subsequent 30-min incubation before infection succeeds in stopping host-specific protein synthesis, so that all protein synthesis postinfection is phage specific. If, however, the cells are not irradiated before infection (Fig. 5), additional proteins can be found associated with the membrane. These are presumably not phage induced; it can be seen that the molecular weights of these proteins (indicated by the lines in Fig. 5) are coincident with those of membrane proteins of uninfected cells.

Effect of T4 infection on bacterial membrane proteins. Since it is known that T4 infection induces many physiological changes in the bacterial membrane, it was of interest to determine whether the distribution of host membrane proteins was altered after infection. The experiment summarized in Fig. 6 compares the bacterial proteins found in membranes from growing cells with membranes from T4-infected cells. No major change can be detected in the distribution of bacterial membranes after infection.

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![Fig. 2](https://jvi.asm.org/Downloaded_from)
A T4 membrane protein is operationally defined as one that is membrane associated after purification from the cell. However, it is difficult to assess how this association is related to function because we have no T4 functions to measure on the membrane. Some proteins are removed by washing with low-ionic-strength buffers and with chaotropic agents, and these may be considered to be ionically bound. It is clear, however, that chaotropic agents can re-
move proteins that are functionally integrated into the membrane. For example, 3-lactate dehydrogenase is solubilized by chaotropic agents (20). Subsequent extraction of guanidine-treated membranes with the detergent Sarkosyl selectively extracts the cytoplasmic membrane proteins without affecting those of the cell wall (1, 12, 13).

We have isolated membranes from T4-infected cells and analyzed their protein content by dodecyl sulfate-polyacrylamide gel electrophoresis. By this method, we have been able to demonstrate, in agreement with our previous work as well as that of others (1, 9, 13, 16, 21, 22, 28, 32), that many phage-induced proteins are associated after infection with the host membrane (Fig. 1-3). We have not identified which genes are responsible for the synthesis of most of the proteins (that in itself is a formidable task, and it is not the purpose of this investigation), but we have indicated the gene number where it has been established. The reader is directed to the studies of others (19, 30), which give the molecular weights of many phage T4 proteins. These can be correlated with the molecular weights given in the figures to identify undesignated proteins. Our studies verify that many of the proteins are associated with the host membrane, as operationally defined.

We have also shown that the synthesis and insertion of these proteins into the bacterial membrane are temporally controlled (Fig. 4).

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**Fig. 4.** Kinetics of synthesis of membrane-associated proteins after T4 infection. UV-irradiated E. coli H-150 cells were infected with wild-type T4 at 37°C. The culture was divided into seven flasks, and a 14C-labeled amino acid mixture was added for the indicated intervals of time. Then, non-radioactive Casamino Acids mixture was added, and the infected cells were incubated for an additional 4 min. The cells were rapidly cooled, each culture was harvested, and the membranes were isolated and analyzed by gel electrophoresis with a 10% polyacrylamide slab gel. (a) Cells labeled 0 to 3 min after infection; (b) 3 to 6 min; (c) 6 to 8 min; (d) 8 to 10 min; (e) 10 to 15 min; (f) 15 to 20 min; and (g) 20 to 25 min.

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**Fig. 5.** Effect of UV irradiation of bacteria on subsequent synthesis of phage membrane proteins. Infection and labeling of E. coli H-150 cells was carried out as described in the legend to Fig. 1. The cells were labeled 2 to 15 min postinfection. Membranes were isolated as described in Materials and Methods and subjected to polyacrylamide (10%) gel electrophoresis. (a) Cells were exposed to UV irradiation and then infected with wild-type T4 as described in the legend to Fig. 1. (b) Cells were not irradiated before infection. (c) Uninfected cells labeled with 14C-amino acid mixture for 15 min during growth.
and that, once in the membrane, these proteins are apparently stably integrated (Fig. 2). It is also important to note that, in agreement with previous work (1, 16), the synthesis of certain host membrane proteins is only slowly shut off after T4 infection (Fig. 5), contrary to what occurs with the majority of the other host proteins (3). In this connection, it has been shown that certain mRNA’s which direct the synthesis of certain outer-wall membrane proteins are extremely long-lived (15), and they presumably can function in the cell even after infection. These proteins are not found labeled in mem-

branes isolated from T4-infected cells that had been exposed to UV irradiation before infection. These results also show that this treatment is useful in inhibiting host functions. It is evident, therefore, that the continued synthesis of these host proteins during infection is not necessary for normal infection.

Lastly, we show that there is no major redistribution or loss of bacterial proteins from E. coli membranes as a consequence of T4 infection (Fig. 6). Therefore, the membrane changes that occur after infection (e.g., increased permeability) are a result of more subtle alterations than can be observed by one-dimensional gel electrophoresis (32).

The fact that so many T4 proteins associate with the host membrane must indicate the importance of the membrane in the maintenance of the infection. However, it is unclear what the significance of this association is.

LITERATURE CITED