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Proteinase Sensitivity of Bacteriophage Lambda Tail Proteins gpJ and pH* in Complexes with the Lambda Receptor

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Previous studies have shown that bacteriophage lambda initially binds to liposomes bearing its receptor protein by the tip of the tail fiber (type 1 complex). It then associates more directly so that the hollow tail tube is in direct contact with the membrane (type 2 complex). DNA can be injected across the lipid bilayer into the liposome from type 2 complexes. We show here that gpJ, the tail fiber protein, becomes more sensitive to proteolytic degradation in type 2 complexes, indicating that the tail fiber does not pass into the liposome and that the tail fiber may undergo a conformational change in type 2 complexes. Another bacteriophage protein, pH*, is sensitive to proteolytic degradation in free bacteriophage, type 1 complexes, or type 2 complexes formed with free receptor, but is resistant to proteinases in type 2 complexes formed with liposomes. This finding suggests that pH* associates with the membrane. We suggest that this association is part of the mechanism by which a transmembrane hole for DNA entry is formed.

Bacteriophage lambda consists of a head of diameter 60 nm containing the 48,502-base pair lambda DNA molecule (17) and a tail through which the DNA passes during infection. The tail consists of a hollow tube 135 nm in length, a conical part 15 nm in length, and a tail fiber 23 nm in length which is attached to the conical part at the distal end of the tail. (For a review of lambda tail structure and assembly, see reference 8). The tail fiber is composed of two to four copies of polypeptide gpJ, which attaches the bacteriophage to the surface of the cell by binding to the outer membrane protein *lamB*, the lambda receptor. (For a review of bacteriophage adsorption, see reference 21). The hollow tube of the tail consists of 32 stacked disks, each of which is formed by six subunits of the major tail protein, gpV, arranged so that each disk has a central, 3-nm-diameter hole (1, 3). The conical part of the tail is apparently composed of three or four disks whose protein composition is not directly known. Assembly of the tail occurs on a structure probably related to the conical part whose formation requires the products of the lambda tail genes J, I, L, K, H, G, and M. gpV polymerizes on the initiator to form the tail tube (3). gpU apparently acts to terminate polymerization at the appropriate length, and gpZ completes the tail in a form that can be attached to the head (6). By gel electrophoresis or complementation, the products of nine lambda tail genes (Z, U, V, G, T, H, M, L, and J) have been shown to be contained in bacteriophage particles.

Since gpJ is the target of neutralizing antibodies, and since host range mutations which alter the specificity of the attachment reaction map within gene J, it is clear that gpJ is the protein which directly binds to *lamB* (2, 11). Antibodies to gpJ have been shown to bind to the tail fiber (S. Kar and R. Hendrix, personal communication), and it is possible that gpJ is the only protein in the tail fiber.

During assembly, gpH (92,294 daltons from the nucleotide sequence, reference 17) is cleaved to form a 78,000-dalton polypeptide called pH* (5). Cleavage of gpH occurs at a step in the assembly pathway between the action of gpU and gpZ (22). Unattached tails thus contain pH*. However, neither pH* nor gpH is found in polytails made by U^- bacteriophage which do contain both the conical part and the tail fiber (6). pH* must play some role in the injection process. *pel* mutants of *Escherichia coli* (18) which bind lambda but do not allow DNA injection to occur can be used to isolate bacteriophage mutants, λhp, capable of injection into pel^- strains. Most of the λhp mutations map in gene H, and a few map in gene V (19).

We have previously visualized two types of complexes of bacteriophage lambda and its receptor by using receptor-bearing liposomes or cell membrane fragments (15). The complexes which form with the *lamB* protein isolated from *E. coli* K-12 are reversible and involve binding only between the tips of the tail fibers and the receptors, so that the hollow tails and the membrane are separated by 17 nm. These complexes are referred to as type 1 complexes. When *Shigella* receptors are used instead of *E. coli* receptors, similar type 1 complexes can be visualized at 4°C. When these complexes containing the *Shigella* receptors are shifted to 37°C, the banded regions of the tails are found to be in direct contact with the surface of the liposome (type 2 complexes), with no gap visible. DNA is injected into the liposomes from type 2 complexes (16). Liposomes therefore provide a realistic model for studying bacteriophage adsorption and DNA injection.

If the tail fibers have been drawn into the liposomes by some type of ratchet mechanism, the hollow tails would be pulled to the surface of the liposomal membranes. This hypothesis would predict that in type 2 complexes, gpJ should be internal and protected by the liposomes from external agents such as proteinases, just as DNA injected into liposomes is protected from DNases (16). We show here that gpJ is not protected, but rather becomes more sensitive to proteolytic degradation in type 2 complexes. An alternative hypothesis in which the conical parts of the tubular tails directly interact with the lipids of the membrane bilayer or the *lamB* protein (or both) might predict that some proteins in the basal part would be protected from proteolytic digestion by insertion into or interaction with the membranes. We show here that pH* is protected from proteolytic cleavage in type 2 but not in type 1 complexes.

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MATERIALS AND METHODS

Formation of complexes. λ*c*I857 S7 bacteriophage were prepared by thermal induction and purified by velocity and equilibrium centrifugation as described previously (15, 16). Viability was approximately 25% as measured by plaque formation and absorbance at 260 nm.

^{35}S-labeled bacteriophage were prepared by growing *E. coli* C600 (λ*c*I857 S7) in M9 minimal medium supplemented with an unlabeled amino acid mixture lacking methionine and cysteine. Lambda development was induced by raising the temperature to 42°C; after 30 to 40 min of growth at 37°C, 10 μCi of [^{35}S]methionine per ml (New England Nuclear Corp.; 1,000 Ci/mmol) was added. Bacteriophage were purified by velocity and equilibrium centrifugation.

Shigella receptor was extracted from *E. coli* Pop 154 which carries the *Shigella lamB* gene introduced by transduction (14) and *E. coli* receptor from strain Yme1 by suspension in 1% cholate–2 mM EDTA–10 mM Tris-hydrochloride (pH 7.5), and receptor-bearing liposomes were prepared as described previously (16). Free receptor was prepared by extracting receptor bearing liposomes with 10 volumes of butanol because the process of incorporating the receptor into liposomes results in a substantial purification. The resulting precipitate was centrifuged, dried in a stream of nitrogen, dissolved in 2% cholate–10 mM Tris-hydrochloride (pH 7.5), and dialyzed against 10 mM Tris-hydrochloride (pH 7.5)–2 mM $MgSO_4$.

Bacteriophage-receptor complexes were formed by mixing 50 μl of receptor or receptor-bearing liposomes in 10 mM Tris-hydrochloride (pH 7.5)–2 mM $MgSO_4$ with 4.8×10^{10} bacteriophage particles. The mixture was incubated at 37°C for 30 min.

Purification of complexes formed with liposomes. After incubating 1.6×10^{11} bacteriophage particles with 200 μl of liposomes for 30 min at 37°C in 10 mM Tris-hydrochloride (pH 7.5)–2 mM $MgSO_4$, 1.0 ml of saturated CsCl was added, giving the sample a final density of ca. 1.7 g/cm^3. These samples were layered under a step gradient consisting of 3.0 ml of CsCl, 1.3 g/cm^3, and buffer, and were centrifuged for 30 min at 45,000 rpm. A liposome or liposome-phage band was then visible at the buffer-CsCl interface, and in samples that contained receptor-free liposomes or liposomes bearing *E. coli* receptor, a bacteriophage band was visible at the interface of the two CsCl solutions. The bands were collected, dialyzed against buffer, and lyophilized before gel electrophoresis.

Purification of complexes with free receptor. Samples were layered onto 10 to 40% sucrose gradients overlying 0.5 ml of CsCl (1.6 g/cm^3) and centrifuged for 90 min at 45,000 rpm in an SW50.1 rotor. Ten fractions of 0.5 ml were collected, dialyzed against water, and lyophilized before gel electrophoresis.

Proteinase treatment. To incubation mixtures were added 1 μg of pronase and proteinase K. Digestion occurred at 37°C for various times, usually 90 min. Then 10 μl of phenylmethylsulfonyl fluoride (10 mg/ml in 95% ethanol) was added. The mixture was incubated for 5 min at room temperature, frozen at −80°C, and lyophilized. In some cases, proteinase-treated bacteriophage were purified by sedimenting in a CsCl step gradient (0.5 ml at 1.6 g/cm^3 and 4.5 ml at 1.3 g/cm^3) for 2 h at 45,000 rpm in the SW50.1 rotor. Fractions were dialyzed against water, lyophilized, and dissolved in 50 μl of sample buffer containing 2 μl of phenylmethylsulfonyl fluoride (10 mg/ml).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The electrophoresis system used was a modification of that described by Laemmli (10) and involved the same buffers. The separating gel consisted of a slab (13.5 cm by 11.0 cm by 0.75 mm) of 10 to 20% (wt/vol) exponential gradient of polyacrylamide formed in an apparatus purchased from R. Shadel (San Francisco, Calif.). The stacking gel was 3.75% (wt/vol) polyacrylamide. The lyophilized samples were dissolved in sample buffer (0.125 M Tris-hydrochloride [pH 6.8]–5% 2-mercaptoethanol–5% sodium dodecyl sulfate–0.0025% bromophenol blue) and placed in a boiling water bath for 45 s. The samples were layered into wells formed in the stacking gel and electrophoresed at 200 V until a marker dye consisting of Coomassie blue R-250 (10 μg/ml) in sample buffer ran off the bottom of the gel.

Autoradiography. Samples prepared for autoradiography contained 200,000 cpm of ^{35}S-labeled bacteriophage at a specific activity of 2×10^{-5} cpm per PFU. After electrophoresis, Kodak X-Omat AR film was exposed to the gel for 2 to 4 days and processed as recommended by Kodak.

Silver staining. Developer solution was prepared by adding to 500 ml of deionized, glass-distilled water 2.5 ml of a freshly prepared 1.0% citric acid solution and 0.25 ml of a 37% solution of formaldehyde. The solution was kept on ice until use. Fixer solution contained 2.5 ml of glacial acetic acid in 500 ml of water. The ammonia-silver solution was prepared within 5 min of use by dissolving one pellet of NaOH in 100 ml of water followed by addition of 2 ml of concentrated NH_4OH and, dropwise, 5 ml of 20% silver nitrate.

A gel, dehydrated overnight in 50% methanol, was placed in a Pyrex dish (20 by 20 cm), and the ammonia-silver solution was added. The dish was agitated continuously on a Thomas rotary shaker under germicidal UV illumination for 15 min. The silver solution was decanted, and the gel was washed four times with water and allowed to rinse with agitation for 5 min longer in 500 ml of water. The water was decanted, and the gel was washed an additional four times with water. The developer was added and decanted when the polypeptide bands reached the desired intensity. The gel was quickly rinsed with water and allowed to sit in fixer solution for at least 15 min.

The gel may be stored wet in fixing solution or may be soaked in fixing solution containing 1.5% glycerol and then dried in a gel drier.

Peptide mapping. Peptide mapping was performed by the limited proteolysis procedure of Cleveland et al. (4). Proteinase-treated or untreated ^{35}S-labeled lambda bacteriophage proteins (10^6 cpm) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Bands of radioactive proteins were cut from the dried gel by using an autoradiogram as a pattern. The slices were incubated in 0.125 M Tris-hydrochloride (pH 6.8)–0.1% sodium dodecyl sulfate–1 mM EDTA for 1 h. The slices were then loaded into the bottoms of wells in a second sodium dodecyl sulfate gel consisting of a 15% separating gel and a 5-cm stacking gel and containing 1 mM EDTA throughout. To the slices were added 10 μl of buffer containing 20% glycerol and then 10 μl of buffer containing 10% glycerol and 0.4 μg of *Staphylococcus aureus* protease V8 (Miles Laboratories, Inc., Elkhart, Ind.). Electrophoresis was begun and then interrupted for 30 min to permit proteolysis when the bromophenol blue tracking dye neared the bottom of the stacking gel. Electrophoresis was continued again until the marker dye reached the bottom of the separating gel. The gel was incubated with an enhancing solution (New England Nuclear), dried, and autoradiographed.

RESULTS

When disaggregated bacteriophage lambda proteins were separated by electrophoresis (Fig. 1, lanes 1 and 12), the major tail proteins visible were gpV (25,814 daltons from the nucleotide sequence), gpJ (124,440 daltons from the nucleotide sequence), and pH* (78,000 daltons), which is the processed form of gpH (92,294 daltons from the nucleotide sequence). The products of six other tail genes (Z, U, G, T, M, and L) have been shown to be present in a few copies per bacteriophage particle, but they are generally not clearly visible on most of these gels because of their relatively low abundance. Several major head proteins including pB*, gpE (38,494 daltons), and gpD (11,574 daltons) are present in large amounts.

Electrophoresis of cholate extracts containing the *Shigella* receptor (Fig. 1, lane 3) and *E. coli* K-12 lambda receptor (Fig. 1, lane 6) reveals numerous bands consistent with the relatively unpurified state of the extract. Preparations of liposomes containing the *Shigella* receptor (Fig. 1, lane 4) and *E. coli* K-12 receptor (Fig. 1, lane 7) have substantially fewer proteins, indicating that most of these contaminating proteins are not efficiently incorporated into the liposomal membrane. The *lamB* protein (47,400 daltons) is a prominent band, especially in gels of *Shigella* receptor-bearing liposomes (Fig. 1, lane 4), showing that incorporation into liposomes results in a substantial purification of *lamB*. The free receptor preparations used in most experiments were extracted from liposomes because of the greater purity of these preparations relative to the crude membrane extracts. Treatment with proteinase resulted in degradation of some of the proteins associated with the liposome but not *lamB* (Fig. 1, lanes 5 and 8). This is especially true in the case of liposomes prepared with the cholate extract of *E. coli* K-12 cells. Reisolation of the proteinase-treated liposomes removes the proteinase (Fig. 1, lanes 5 and 9) and results in a somewhat cleaner liposome preparation whose predominant protein species is *lamB*.

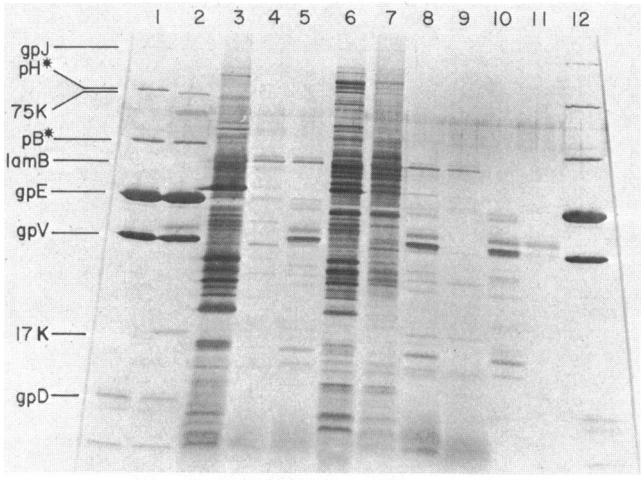

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of bacteriophage lambda and lambda receptor preparations. Lanes (1) structural proteins of bacteriophage lambda; (2) bacteriophage lambda incubated for 3.5 h with pronase and proteinase K; (3) *Shigella lamB* preparation made by cholate extraction; (4) liposomes made in the presence of the *Shigella lamB* preparation; (5) liposomes bearing the receptor from *Shigella* incubated for 3.5 h with pronase and proteinase K; (6) *E. coli lamB* preparation made by cholate extraction; (7) liposomes made in the presence of the *E. coli lamB* preparation; (8) liposomes bearing the *E. coli* receptor incubated for 3.5 h with pronase and proteinase K; (9) liposomes bearing the *Shigella* receptor incubated with proteinases and then repurified on Sepharose 4B; (10) pronase and proteinase K alone, unincubated; (11) pronase and proteinase K alone, incubated for 3.5 h; (12) structural proteins of bacteriophage lambda.

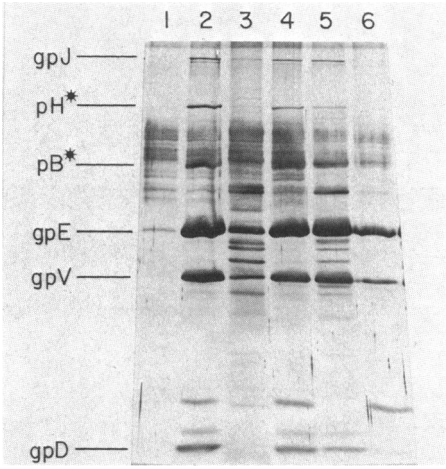

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified complexes formed between bacteriophage and liposomes. Complexes were purified by flotation through a CsCl step gradient as described in the text. Portions of the top and bottom of the gradients containing liposome-bacteriophage complexes or free bacteriophage, respectively, were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Top (lane 1) and bottom (lane 2) fractions of the gradient were used to purify bacteriophage incubated with liposomes not containing receptor. Top (lane 3) and bottom (lane 4) fractions of the gradient were used to purify bacteriophage incubated with liposomes bearing *E. coli* receptor (type 1 complex formation). Top (lane 5) and bottom (lane 6) fractions of the gradient were used to purify bacteriophage incubated with liposomes bearing *Shigella* receptor (type 2 complex formation.

Binding of bacteriophage to receptor-bearing liposomes did not result in molecular weight changes for any of the major lambda head or tail proteins. After incubation of the bacteriophage and liposomes, the complex of bacteriophage and liposomes was allowed to float upward through a CsCl gradient. Most of the added bacteriophage bound to the *Shigella*-bearing liposomes and were found at the top of the gradient. No lambda proteins were completely lost as a result of complex formation with *Shigella* receptor, but the relative amount of pH* was considerably reduced (Fig. 2, compare lanes 2 and 5), as previously reported by Roa (13). Using liposomes bearing the *E. coli* receptor, most of the bacteriophage were left behind (Fig. 2, lane 4), although some were found in the liposome fraction (Fig. 2, lane 3). Since complexes formed with *E. coli* receptor are reversible, this finding indicates that complexes formed with liposomes bearing the *E. coli* K-12 receptor dissociate before they can be separated. Complexes between lambda and the *E. coli* receptor are known to be dissociated by high salt concentrations (20). When liposomes not containing receptor were used, bacteriophage proteins did not become associated with the liposomes to any significant extent (Fig. 2, lane 1). We also showed that proteins from disrupted or disaggregated bacteriophage did not become associated with the liposomes even when added to the lipid before formation of the liposomes, indicating that none of the proteins had a high propensity to become entrapped in membranes (data not shown).

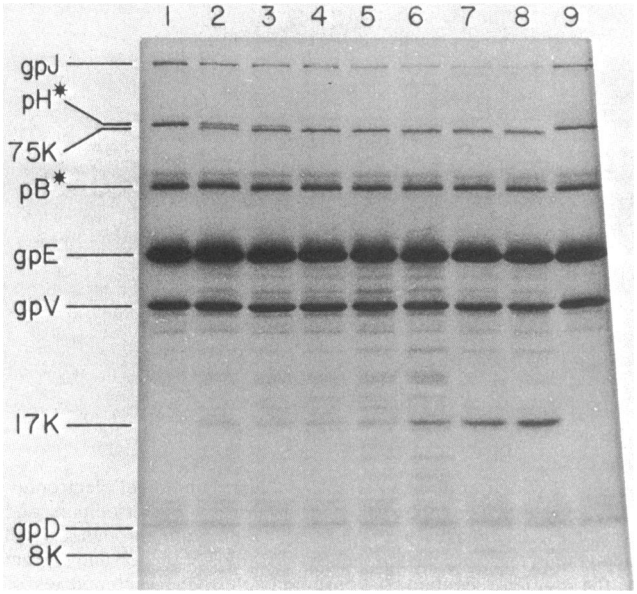

FIG. 3. Time-dependent proteolysis of bacteriophage lambda. Constituent proteins of bacteriophage incubated in the absence of proteinases are shown in lanes 1 and 9. Other lanes show effects of incubation with proteinases. Lanes and incubation times: (2) 1 min; (3) 2 min; (4) 6 min; (5) 15 min; (6) 90 min; (7) 180 min; (8) 270 min. Proteolysis was stopped by additions of phenylmethylsulfonyl fluoride, and gel electrophoresis and autoradiography were performed as described in the text.

No molecular weight changes occurred in bacteriophage proteins when the bacteriophage were incubated with free *Shigella* receptor. After binding, the phage-receptor complex was sedimented through a 10 to 40% (wt/wt) sucrose gradient. The various gradient fractions were then analyzed by gel electrophoresis (not shown). Most of the bacteriophage were found in association with *lamB* near the bottom of the gradient. gpJ and pH* were associated with the fast-sedimenting complexes. Small amounts of gpE and gpV were found in the top fraction not sedimenting with the bacteriophage, but this was seen with control bacteriophage sedimented in the absence of receptor (not shown). The top fractions did not contain detectable amounts of pH* or gpJ.

Incubation of lambda bacteriophage with 20 μg of pronase and 20 μg of proteinase K per ml for 15 min or less did not result in measurable loss of viability. Further incubation with proteinases resulted in a time-dependent loss of viability of 40% after 90 min and 66% after 170 min. Electron microscopic examination of the bacteriophage after 270 min of incubation demonstrated that ejection of DNA had occurred in approximately 50% of the bacteriophage particles.

Electrophoretic analysis of bacteriophage proteins, using autoradiography of ^{35}S-labeled proteins (Fig. 3 and 4) or silver staining of unlabeled proteins (Fig. 1 and 2), revealed several changes which occur in the profiles as a result of proteinase treatment. First, pH* is very sensitive to proteinases and disappears within 15 min (Fig. 1, lane 2, and Fig. 3, lanes 1 to 4). Concomitantly, a band designated 75K, which is smaller than pH* by approximately 3,000 to 4,000 daltons, appeared. This result suggests that a short polypeptide fragment has been cleaved from pH*, a conclusion which was confirmed by cutting pH* and 75K from gels, partially digesting them with protease V8, and comparing the bands generated from each by gel electrophoresis (Fig. 5). Second, the intensity of the gpJ band is very gradually reduced over a 270-min period (Fig. 3, lanes 1 to 8). Third, two new relatively faint bands with molecular weights of ca. 17,000 and 8,000 daltons (17K and 8K) appear, beginning at the earliest times (1 min) and increasing in intensity over the 270-min incubation.

Bacteriophage bound to liposomes bearing *E. coli* receptor (Fig. 4, lane 4) or to free *E. coli* receptor (Fig. 4, lane 6) are affected by proteinases in the same way as free bacteriophage. Most proteins, including gpJ, continued to be resistant to proteolysis, except for pH*, which disappeared and was replaced by the shorter polypeptide 75K. The 17K and 8K proteins also appeared with incubation.

Results which differed in several important respects were obtained when *Shigella* receptor was used. After incubation of bacteriophage with free *Shigella* receptor, gpJ was much more sensitive to degradation (Fig. 4, lane 5). pH* was still very sensitive to the proteinase, so that the band almost disappeared. Appearing at the same time was a faint 75K band and a more intense 72K band which, however, was not as intense as pH* in the free bacteriophage. With liposomes bearing the *Shigella* receptor, gpJ was sensitive to proteolysis, but pH* was highly resistant to proteolysis (Fig. 4, lane 3). Only faint 75K and 72K bands could be detected. The 17K and 8K polypeptides were again present.

Thus, when liposomes bearing the *E. coli* receptor were used, gpJ was resistant to proteinases, but when liposomes bearing the *Shigella* receptor were used, gpJ was sensitive. When liposomes bearing the *Shigella* receptor were used, pH* was resistant to proteinases, but when liposomes bearing the *E. coli* receptor were used, pH* was sensitive. These differences presumably indicate that the proteins are exposed differently in type 1 and type 2 complexes.

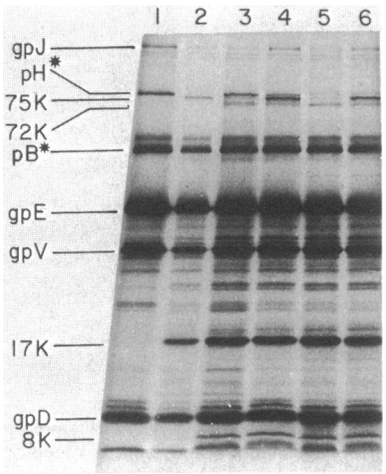

FIG. 4. Effect of proteinases on bacteriophage in complexes with *Shigella* or *E. coli* K-12 receptors in the presence or absence of a liposomal membrane. Complex formation, using ^{35}S-labeled phage and proteinase treatment, was performed as described in the text. Lanes: (1) control of untreated, free bacteriophage; (2) bacteriophage treated with proteinases for 90 min; (3) bacteriophage complexes with liposomes bearing the *Shigella lamB*; proteinase treatment was for 90 min; (4) bacteriophage in complexes with liposomes bearing *E. coli* K-12 *lamB*; proteinase treatment was for 90 min; (5) bacteriophage in complexes with free *Shigella* receptor; proteinase treatment was for 90 min; (6) bacteriophage in type 1 complexes with free *E. coli* K-12 receptor. Proteinase treatment was for 90 min.

DISCUSSION

Most types of bacteriophage are extremely resistant to degradative enzymes such as DNases or proteinases and also to inactivation by adverse physical conditions, including high ionic strengths and prolonged storage. We found that after prolonged incubation with the proteolytic enzymes used here, only approximately 50% of the viable lambda bacteriophage were inactivated, and there was no significant loss of viability during short periods of incubation.

Despite this retention of viability, the band corresponding to the bacteriophage tail protein pH* (78,000 daltons) was quantitatively eliminated after a brief incubation with proteinases. Simultaneously, a new band corresponding to a slightly smaller polypeptide designated 75K appeared. When radioactive bacteriophage were used, the new protein 75K was radioactive, so 75K must be derived from a larger lambda protein. Only gpJ and pH* are larger than 75K, and as the kinetics of appearance of 75K match the kinetics of disappearances of pH* and not gpJ, it is likely that the proteinase cleaves a short polypeptide from pH* to form 75K. More direct evidence based on partial proteolytic digests of 75K and pH* indicate that 75K and pH* are closely related. Cleavage of pH* is not likely to be responsible for the slight loss of viability observed, since approximately 50% of pH* is cleaved within 1 min and all of it is cleaved within 5 min, whereas there was no significant loss of viability for at least 15 min.

Also, a new 17,000- and a new 8,000-dalton polypeptide appeared after proteinase treatment. The 17K protein remained associated with the bacteriophage, but the 8K protein did not. Possibly, both of these polypeptides were formed from gpV. From the intensities observed on the autoradiogram or the stained gels, only the head protein gpE or the tail protein gpV was abundant enough to account for the amount of the 17,000-dalton polypeptide ultimately observed. We found that the 17K and 8K fragments could be produced by proteolysis of purified tails alone (data not shown), so gpV is a likely candidate, especially since Katsura (7) has shown that disks of the tail tube (composed of gpV) have small knobs protruding from the core. Deletions mapping within gene V are known to reduce the size of gpV to polypeptides as short as 18,000 daltons. Bacteriophage carrying these deletions are viable and apparently lack the knobs, suggesting that a 7,000-dalton segment of the gpV polypeptide is dispensible and might be exposed. It seems likely that proteolytic treatment degrades some of the knobs, leaving behind a protein of 17,000 daltons in the bacteriophage tail and releasing an 8,000-dalton fragment.

After prolonged incubation, the intensity of the band corresponding to gpJ declines. Possibly, the decline in phage viability and the increase in the number of bacteriophage with empty heads observed after proteinase treatment is the result of proteolytic damage to the tail fiber which results in DNA ejection. Supporting the idea that DNA ejection is somehow triggered by alterations in the tail fiber is the fact that host range mutants or lambda (λh) which map in gene J cause the λh bacteriophage to be less stable and also to eject their DNA under conditions in which λh^+ does not do so (12). Alternatively, proteinase treatment could trigger DNA ejection in some other way, and ejection of the DNA could subsequently render gpJ more sensitive to proteolytic attack.

We have examined the proteolytic sensitivities of these proteins in complexes of lambda bacteriophage with the *E. coli* and *Shigella* receptors. In the absence of proteinases, formation of bacteriophage-receptor complexes does not alter the molecular weight profile of the polypeptides. After addition of proteinases to type 1 complexes formed with free *E. coli* receptors or with liposomes bearing the *E. coli* receptor, the electrophoretic profile of polypeptides does not markedly differ from that seen after proteinase treatment of free bacteriophage. In particular, gpJ is relatively resistant to proteolytic degradation, both in free bacteriophage and in type 1 complexes. pH* is largely replaced by 75K and by a faint new band designated 72K, although some of the pH* band remains. Because complex formation with *E. coli* receptors is reversible, the conversion of pH* to 75K may not be especially significant, since this could occur either in the complex or on free phage that have disassociated from the complex. We can, however, at least conclude that formation of type 1 complexes does not render gpJ exceptionally sensitive to proteolytic degradation.

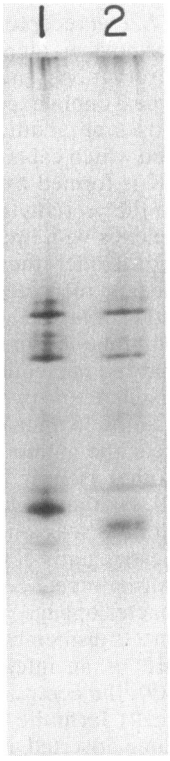

FIG. 5. Autoradiogram of the peptide fragments created by limited proteolysis indicates close correspondence of the fragments from pH* (1) and the 75K protein (2). Differences presumably reflect the presence of ca. 3,000 daltons of amino acid sequence in pH* not present in 75K.

Addition of proteinase to complexes formed with *Shigella* receptor gives a different result. gpJ was shown to be very susceptible to degradation in complexes formed either with free *Shigella* receptor or with liposomes bearing *Shigella* receptor. The latter finding indicates that gpJ does not enter the liposomes and become protected after formation of type 2 complexes, but instead remains outside in an exposed configuration. The nature of the configurational change that occurs in the tail fiber to render it susceptible to proteolytic attack is at present unknown, but it may be significant that the bacteriophage DNA is ejected in type 2 complexes.

In complexes with free *Shigella* receptor, pH* disappears and is replaced by 75K, with a trace of 72K (Fig. 4, lane 5) visible; 72K is also detected (Fig. 4, lane 3) in minor amounts

in complexes with free *E. coli* receptor. However, 72K is not seen when free bacteriophage alone are incubated with proteinases. These observations suggest that is some involvement of pH* in type 2 complexes involving free *Shigella* receptor which exposes an additional cleavage site. An alternative interpretation which cannot be ruled out by these experiments is that 72K is formed by cleavage by gpJ.

A major difference in the proteolytic susceptibility of pH* is observed when complexes with liposomes bearing *Shigella* receptor are formed. Under these conditions, pH* is almost completely resistant to proteolysis. No 75K is observed, and there is rather little 72K. We conclude that the presence of a lipid membrane in type 2 complexes protects pH*. It is of interest that the nucleotide sequence of gene H (17) demonstrates existence of two segments in the H polypeptide, each containing 35 uncharged amino acids. One of these segments is near the amino terminus (amino acids 113 to 145), and the other is near the carboxyl terminus (amino acids 697 to 732). Neither segment is sufficiently near the end of gpH to result in its loss when gpH is processed to pH*. One or both of these regions of pH* may penetrate the liposomal membrane during type 2 complex formation, possibly anchoring the bacteriophage to the membrane, or possibly helping to form a transmembrane pore for the DNA. If, as seems likely, pH* is an integral component of the conical part of the tail (9), the central hole of the last disk of the tail could automatically form the transmembrane pore if the terminal disk became inserted into the membrane by interactions between membrane lipids and pH*.

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