JOURNAL OF VIROLOGY, Sept. 1975, p. 685-695
Vol. 16, No. 3

Proteins of Bacteriophage ϕ6

JACQUELINE FRANZESE SINCLAIR, ALEXANDER TZAGOLOFF, DOROTHY LEVINE, AND LEONARD MINDICH*

The Public Health Research Institute of the City of New York, Inc., New York, New York 10016

Received for publication 3 April 1975

We investigated the protein composition of the lipid-containing bacteriophage ϕ6. We also studied the synthesis of phage-specific proteins in the host bacterium *Pseudomonas phaseolicola* HB10Y. The virion was found to contain 10 proteins of the following molecular weights: P1, 93,000; P2, 88,000; P3, 84,000; P4, 36,800; P5, 24,000; P6, 21,000; P7, 19,900; P8, 10,500; P9, 8,700; and P10, <6,000. Proteins P3, P9, and P10 were completely extracted from the virion with 1% Triton X-100. Protein P6 was partially extracted. Proteins P8 and P9 were purified by column chromatography. The amino acid composition of P9 was determined and was found to lack methionine. Labeling of viral proteins with [^{35}S]methionine in infected cells indicated that proteins P5, P9, P10, and P11 lacked methionine. Treatment of host cells with UV light before infection allowed the synthesis of P1, P2, P4, and P7; however, the extent of viral protein synthesis fell off exponentially with increasing delay time between irradiation and infection. Treatment of host cells with rifampin during infection allowed preferential synthesis of viral proteins, but the extent of synthesis also fell off exponentially with increasing delay time between the addition of rifampin and the addition of radioactive amino acids. All of the virion proteins were seen in gels prepared from rifampin-treated infected cells. In addition, two proteins, P11 and P12, were observed; their molecular weights were 25,200 and 20,100, respectively. Proteins P1, P2, P4, and P7 were synthesized early, whereas the rest began to increase at 45 min post-infection.

Bacteriophage ϕ6 is unique in that it contains three pieces of double-stranded RNA and an envelope composed of proteins and lipids (16). The virion has been shown to consist of a polyhedral core and a membranous envelope structure (16). The RNA molecules have molecular weights of 2.2×10^6, 2.8×10^6, and 4.5×10^6 (11). The virion has 25% lipid, 13% RNA, and 62% protein (16). The development of the virus takes place in the interior of the host cell and appears to be localized within the nucleoplasms of the cell (3).

Another lipid-containing bacteriophage, PM2, had been isolated earlier (4). This virus infects a marine species of *Pseudomonas* and has a structure quite different from that of ϕ6. PM2 has a genome consisting of a circular double-stranded molecule of DNA of 6×10^6 daltons (5), and the virion contains four proteins (7) and about 13% lipid (1).

ϕ6 should be extremely useful in elucidating the interactions between lipids and proteins in membrane biogenesis as well as genetic and biochemical problems involving double-stranded RNA genomes.

In this report we present our findings on the characteristics of the viral proteins and procedures for studying their in vivo synthesis.

MATERIALS AND METHODS

Bacterial strains and media. *Pseudomonas phaseolicola* HB10Y and bacteriophage were obtained from A. Vidaver (16). Strain HB(RP1) is a derivative of HB10Y that carries a drug resistance plasmid, RP1 (6). Leucine and methionine auxotrophs were obtained after mutagenesis with ethyl methane sulfonate and replica plating.

Media used were: M8, containing 3 g of Na_2HPO_4, 1.5 g of KH_2PO_4, 25 g of NaCl, 0.5 g of NH_4Cl, and 50 mg of $MgSO_4$ per liter, pH 6.5; and LC, containing 10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter, pH 7.0.

Preparation of [^{14}C]leucine-labeled ϕ6. *P. phaseolicola* strain HB10Y was grown in M8 minimal medium supplemented with 0.4% glucose, 2 mM $MgSO_4 \cdot 7H_2O$, 1 mM $CaCl_2$, 200 μg of glutamic acid per ml, and phenylalanine, serine, methionine, and histidine at 20 μg/ml. The cells were infected at a density of 2.8×10^8/ml with ϕ6 at a multiplicity of infection (MOI) of 0.1. After 140 min of further incubation, [^{14}C]leucine (New England Nuclear Corp.; 298 mCi/mmol) was added to 3 μCi/ml and the incubation continued until lysis occurred. Cell debris was removed by low-speed centrifugation, and the

virus was treated with 10% (wt/vol) polyethylene glycol (Carbowax 6000) in the presence of 0.5 M NaCl for 15 min at 0 C. The virus was pelleted by centrifugation at 10,000 rpm for 10 min and resuspended in buffer A (16). This viral suspension was then layered on a 10 to 30% sucrose gradient and centrifuged for 75 min at 23,000 rpm. Fractions were collected and the viral band was centrifuged overnight in a 30 to 60% linear sucrose gradient. The viral band was removed with a syringe diluted with buffer A and pelleted by centrifugation at 35,000 rpm for 120 min.

Determination of molecular weights of viral proteins. The proteins of radioactive virus and infected cells were analyzed by discontinuous sodium dodecyl sulfate (SDS)-polyacrylamide slab gel electrophoresis (10, 13), using a 5% spacer gel and a 10 or 15% separating gel. The gels were 1 mm thick and either 10 or 15 cm long. One part of a mixture of 150 mM Tris-hydrochloride (pH 6.8), 3% SDS, 3% mercaptoethanol, 6 mM EDTA, and 30% glycerol was added to 2 parts purified radioactive virus and heated in boiling water for 1 min immediately and/or before application to the gel. Approximately 25,000 counts/min of ^{14}C in a volume of 10 μl was applied to each well, and the gel was run at 17-mA constant current. Positions of the viral proteins were determined by autoradiography on Kodak no-screen X-ray films (NSZT). Protein markers used in estimation of the molecular weights of viral proteins consisted of phosphorylase A (94,000 mol wt), bovine serum albumin (68,000 mol wt), ovalbumin (43,000 mol wt), carbonic anhydrase (29,000 mol wt), soybean trypsin inhibitor (21,000 mol wt), myoglobin (17,200 mol wt), cytochrome *c* (11,700 mol wt), and insulin (2 subunits of 3,000 and 2,700 mol wt). The positions of the marker proteins were determined by staining the gel in 1% amido black solution in a solvent consisting of methanol-water-acetic acid (5:5:1) for 15 min at room temperature. The gel was destained in the same solvent without dye and then swelled to original size in 7% acetic acid.

Labeling of viral proteins in infected cells: UV-irradiated cells. Incorporation of radioactive amino acids was low in UV-treated cells, and therefore amino acid auxotrophs were used as host cells. This was not necessary in the case of untreated or rifampin-treated cells. A leucine auxotroph of HB(RP1) was grown in M8 supplemented as before with the addition of 15 μg of $FeSo_4 \cdot 7H_2O$ and 20 μg of L-leucine per ml. At a concentration of 3.2×10^8/ml, the cells were washed twice and resuspended in the same medium without leucine. Ten milliliters of resuspended culture was transferred to a 9-cm petri dish and irradiated for 4 min at 1,500 ergs/mm^2 per min with continuous shaking. The UV-treated culture was incubated in a tin foil-covered test tube or flask for 2.5 min (unless indicated otherwise) at 26 C with shaking.

ϕ6 was added at MOI 20, and at 10 min postinfection [^{14}C]leucine was added to give 6 μCi/ml. The labeling was continued until 85 min postinfection, at which time the sample was diluted 1:6 into prewarmed LC (26 C) for 5 min and then transferred to an ice bath to arrest both the infection and labeling. An aliquot was removed to determine the trichloroacetic acid-insoluble radioactivity, and the remaining sample was centrifuged, resuspended in 0.04 volume of electrophoresis sample buffer (13), and heated for 1 min in boiling water immediately and/or before application to the gel. All samples were stored by freezing. Infection of UV-irradiated cells in the presence of [^{35}S]methionine was carried out by using a methionine auxotroph of HB(RP1).

Infected cells with rifampin. HB was grown in M8 supplemented as given before with the addition of 15 μg of $FeSO_4 \cdot 7H_2O$ per ml. At a concentration of 3.2×10^8/ml, the cells were infected with ϕ6 at MOI 50. In the rifampin-treated cultures, the antibiotic was added to infected cells at 60 min postinfection at a final concentration of 50 μg/ml. [^{14}C]leucine (Schwarz/Mann; 312 mCi/mmol) was added at final concentration of 2 μCi/ml at various times after the addition of rifampin. After 15 min of labeling, the culture was chilled and transferred to cold LC (0 C). At this point, the procedure was the same as that given for the irradiated cells.

In infected cells to which no antibiotic was added, the procedure was essentially the same as the rifampin-treated cells, except that [^{14}C]leucine (2 μCi/ml) was added to the culture at various times after addition of virus, and the labeling continued until 90 min postinfection.

All labeled proteins in infected cells were analyzed on 15% discontinuous SDS-acrylamide slab gels, using the same system as that given for the purified virus. The relative amounts of viral proteins in individual bands were estimated by determining the area of the peaks obtained by scanning the autoradiograms with a Joyce-Loebl microdensitometer.

Extraction and chromatography of viral proteins. Purified virus prepared according to Vidaver et al. (16) was mixed with purified [^3H]leucine-labeled virus so as to give 7.74 mg of virus and 6.8×10^5 counts/min in 2 ml of buffer A. The suspension was made 1% in Triton X-100 and centrifuged for 1 h at 50,000 rpm in the cold. The supernatant liquid had 54% of the total radioactivity. The supernatant was applied to a column (1 by 10 cm) of Whatman DE-52 equilibrated with 0.2% Triton X-100, 5 mM $NaPO_4$, pH 7.5. The column was washed with 10 ml of 0.2% Triton X-100, 5 mM $NaPO_4$, and then a linear gradient consisting of 60 ml of 5 to 100 mM phosphate in 0.2% Triton X-100 was applied. The fractions were analyzed electrophoretically, using the disc gel system of Weber and Osborn (18) with 7.5% acrylamide.

The pellet from the Triton X-100 extraction was dissolved in 2 ml of 6 M guanidine hydrochloride and centrifuged at 50,000 rpm for 30 min, and the supernatant was dialyzed in 0.25-inch (about 0.62 cm) Visking tubing for 90 min in 1 liter of water. A precipitate formed which was collected by centrifugation at 10,000 rpm for 10 min, washed with 5 ml of water, and dissolved in 2 ml of 8 M urea, 5 mM sodium phosphate, pH 7.5. This fraction, which constituted 42% of the original radioactivity, was applied to a column (1 by 10 cm) of Whatman DE-52 equilibrated with 8 M urea, 2 mM sodium phosphate, pH 7.5. The column was washed with 10 ml of the

same buffer, and then a 60-ml linear gradient of 2 to 100 mM sodium phosphate, pH 7.5, in 8 M urea was applied.

Amino acid analysis of P9. The protein of fractions 5 to 8 in Fig. 8 was precipitated with 90% acetone and washed several times with water to remove acetone, and 250 μg was hydrolyzed in 0.5 ml of 6 N HCl + 2% mercaptoethanol for 24 h at 105 C. Duplicate samples representing 35 μg were analyzed with a Beckman model 120 C amino acid analyzer.

Electron microscopy. Samples of φ6 (4.8 mg/ml) in 5 mM Tris-acetate, pH 7.5, were treated with Triton X-100 at 0 C. A drop was applied to carbon-coated grids and excess fluid was removed with filter paper. A drop of 2% phosphotungstic acid pH 7.0, was applied and excess fluid was removed. The grids were examined with a Philips 300 electron microscope.

RESULTS

Identification of viral proteins in virions and infected cells. Purified virus gave a pattern with 10 bands after electrophoresis in SDS-slab gels (13) (Fig. 1a). These proteins have been designated P1 to P10. The pattern was similar for unlabeled virus and for virus labeled with [^{14}C]leucine. The molecular weights of the proteins were estimated from their mobilities in gels as described in Materials and Methods relative to the mobilities of proteins of known molecular weight (18) using both the electrophoretic systems of Weber and Osborn (18) and Studier (13). Table 1 shows the molecular weights of the virion-specific proteins as well as their relative amounts in the virus article. The estimation of composition assumed a uniform weight fraction of leucine in each protein and was therefore somewhat imprecise.

Labeling of infected cells with [^{14}C]leucine gave a complex pattern in which viral proteins were obscured by a heavy background of host proteins. If, however, rifampin was added to infected cells before the addition of radioactive amino acid, then a rather simple pattern was obtained (Fig. 1c). The 10 virion proteins were observed as well as two additional proteins designated P11 and P12. Uninfected cells treated similarly with rifampin incorporated more radioactivity into host proteins than did infected cells (Fig. 1b); however, they did show a diminution in incorporation of radioactive amino acids relative to untreated cells. The relative amounts of the virus-specific proteins in infected cells is shown in Table 1, as well as the molecular weights of P11 and P12. When infected cells were treated with rifampin and labeled with [^{35}S]methionine, a pattern was obtained that was similar to that found with radioactive leucine, except that proteins P5, P9, and P10, and P11 were not labeled (Table 1).

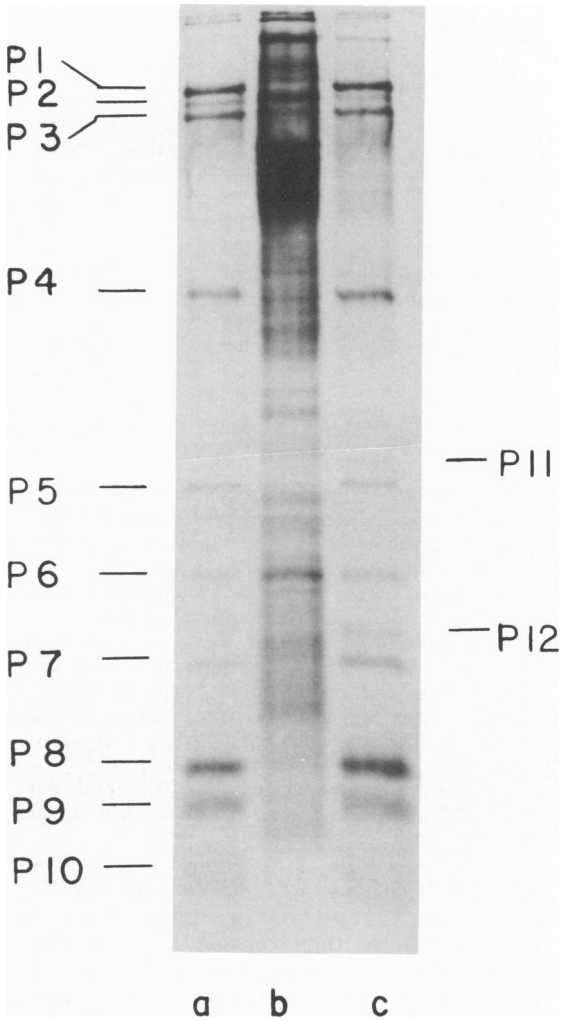

FIG. 1. *Autoradiograph of φ6 proteins in virions and infected cells. Electrophoresis was in a 15% discontinuous polyacrylamide slab gel, as described in the text. The autoradiogram was exposed for 2 days; the origin is at the top of the pattern. Cells, grown in supplemented M8, were infected with φ6 at MOI 50. At 60 min after infection, rifampin was added to a final concentration of 50 μg/ml. Incubation was continued for 10 min, followed by the addition of [^{14}C]leucine to 2 μCi/ml. (a) Purified φ6, isolated as described in the text; (b) uninfected cell lysate; (c) φ6-infected cell lysate.*

Inhibition of viral protein synthesis by rifampin. Although virus-specific protein synthesis could be observed in cells treated with rifampin (Fig. 1), the extent of this synthesis decreased with increasing time of exposure of rifampin. Figure 2 illustrates the extent of synthesis of P4 and P5 when [^{14}C]leucine was added at various times after the addition of

TABLE 1. *Characteristics of φ6 proteins*

Protein	Mol wt[a]	Total pure virus (%)	Total infected cells[b] (%)	Extractable by 1% Triton X-100[c]	Containing methionine[d]
P1	93,000	15.6	13.3	–	+
P2	88,000	6	3.7	–	+
P3	84,000	9.8	12.3	+	+
P4	36,800	6.8	9.1	–	+
P5	24,000	3	2.7	–	–
P6	21,000	2	3.5	±	+
P7	19,900	2.9	6.9	–	+
P8	10,500	26.3	26.7	–	+
P9	8,700	18	12	+	–
P10	3,000–6,000	9.5	6.8	+	–
P11	25,200		0.6		–
P12	20,100		0.2		+

[a] Determined by comparing the electrophoretic migration of φ6 proteins with marker proteins in SDS-acrylamide slab gels as described in the text.

[b] Estimated from a Joyce-Loebl microdensitometer recording of the autoradiogram shown in Fig. 1.

[c] Extraction of φ6 with 1% Triton X-100 was as described in the text, using both unlabeled and labeled virus and analyzing the extracts and remainders on SDS-gels according to Studier (13) and Weber and Osborne (18). Symbols: +, Completely extractable; ±, partially extractable.

[d] Infected cells were labeled with [^{35}S]methionine and the protein was analyzed as described in the text.

rifampin. It can therefore be concluded that the synthesis of viral proteins is sensitive to rifampin but less so than that of host proteins. Although the decay of synthesis of only proteins P4 and P5 is shown, the synthesis of all viral proteins followed the same pattern.

Inhibition of viral protein synthesis by UV irradiation. When host cells were irradiated with UV light before infection with φ6, the synthesis of host proteins was markedly reduced. The synthesis of phage-specific proteins was also reduced compared with untreated or rifampin-treated cells; however, the most striking effect was complete inhibition of the synthesis of most viral proteins. Only proteins P1, P2, P4, and P7 were synthesized under these conditions (Fig. 3), and the synthesis of these four proteins was linear over 2 h (results not shown). The band representing P2 is apparent in the original autoradiograms but is difficult to reproduce photographically. A very light band at the position of P3 appeared in some of the experiments, especially after labeling periods of over 60 min. Identical results were obtained whether cells were labeled with either [^{14}C]leucine or [^{35}S]methionine (Fig. 3).

In addition, the extent of labeling of the above four proteins diminished with increasing time between irradiation and infection. The decrease in the ability to form P4 is illustrated in Fig. 4. These results are consistent with there being a requirement for a labile host product for initiation of viral protein synthesis as well as the existence of at least two classes of viral proteins, perhaps early and late.

Pulse labeling of viral proteins. When infected cells were labeled for 2-min intervals at various times during the infection cycle, the earliest proteins to appear corresponded to those formed after UV irradiation (Fig. 5 and 6). Synthesis of proteins P1, P2, P4, and P7 began at approximately 10 min after infection; however, the other proteins did not appear until about 45 min after infection (Fig. 5 and 6). The background of host protein synthesis diminished somewhat at the same time that the late proteins began to increase (Fig. 1 and 5). This decrease in host synthesis was not observed in cells not treated with rifampin. The labeled material that was observed between P3 and P4 (Fig. 1c) appeared to be host proteins since it corresponded to intense bands seen in uninfected cells (Fig. 1b) and diminished in intensity with later labeling times (Fig. 5). The band

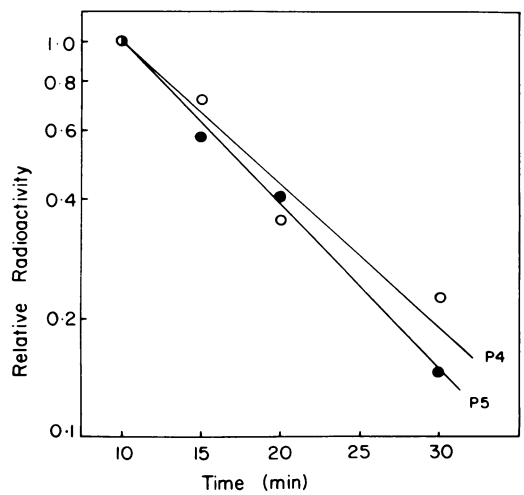

FIG. 2. *Effect of rifampin on viral protein synthesis. Cells were infected with φ6 at MOI 50, and rifampin was added at 60 min after infection. At different time periods after the addition of rifampin, the cells were labeled for 15 min with 2 μCi of [^{14}C]leucine per ml. Samples were prepared and subjected to electrophoresis as described in the text. The relative amounts of the individual proteins were estimated from a Joyce-Loebl microdensitometer tracing of the autoradiogram. The results for proteins P4 and P5 are shown.*

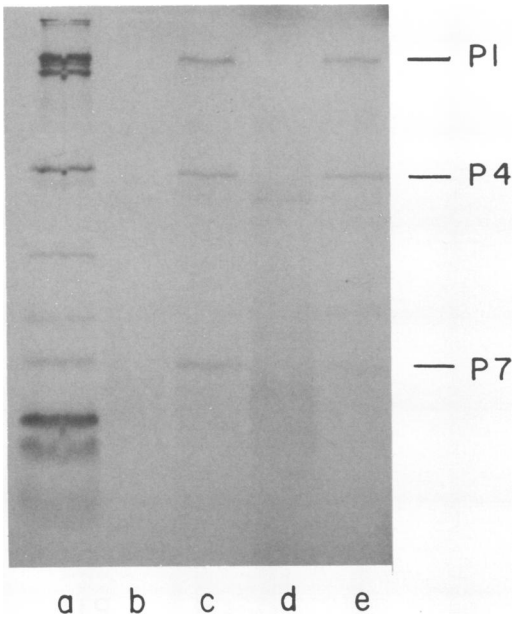

FIG. 3. *Autoradiograph showing synthesis of φ6 proteins in UV-irradiated cells. Cells were irradiated with UV light as described in the text. Virus was then added at MOI 20. At 10 min after infection, the appropriate radioactive amino acid was added to the cells, and the labeling continued until 85 min after infection. (a) Purified virus; (b) a leucine auxotroph of HB(RP1) labeled with [^{14}C]leucine; (c) the same culture infected with φ6; (d) a methionine auxotroph of HB(RP1) labeled with [^{35}S]methionine, (e) the same culture infected with φ6.*

for P8 in Fig. 5 was a doublet due to the appearance of a new species of mol wt 11,600. Since the two bands together had twice as much material as P9, which was identical to that found in cells pulsed for longer periods of time (Fig. 1, Table 1), and since the upper band was not seen in the longer pulses, we concluded that this was a precursor to P8.

Extraction and purification of viral proteins. φ6 was inactivated by exposure to Triton X-100 (16). Exposure to this detergent led to the disruption of the outer envelope of the virus and ultimately to its removal (Fig. 7). Forty-seven percent of the viral protein, as measured with the Folin reagent (9), was removed by treatment with a solution of 0.5% Triton X-100, and 54% was removed with a 2% solution. The extractable material contained proteins P3, P6, P9, and P10. Figure 8A, B, and D shows the extraction of proteins P3 and P9. Protein P9 had been purified from the Triton extract, as described in Materials and Methods and illustrated in Fig. 8C and Fig. 9. Its amino acid composition was determined and is shown in Table 2. The protein was not particularly hydrophobic as indicated by its composition, but it lacked methionine. The latter has been confirmed by lack of methionine incorporation into the protein in labeling studies. Protein P8 had also been purified by extracting the residue after Triton extraction with guanidine hydrochloride, as described in Materials and Methods and illustrated in Fig. 8E and Fig. 10.

DISCUSSION

The results presented in this paper demonstrate 12 viral proteins, as resolved by SDS-acrylamide gel electrophoresis, that are synthesized in cells infected by φ6 (Fig. 1c). Ten of these proteins are found in purified φ6 virions (Fig. 1a). The total molecular weight of all 12 proteins is 437,000. The structural genes for these proteins account for approximately 86% of the viral genome. We have, therefore, identified most or all of the virus-specific proteins. During a 2-min pulse with radioactive leucine, two bands appear in the region of viral protein P8 (Fig. 5). However, in both the pure virus and under conditions of 10- or 15-min pulse labelings (Fig. 1a and 1c), there is only one band, P8, whose extent of labeling approximates the sum of the two bands in the shorter pulse. Thus, P8

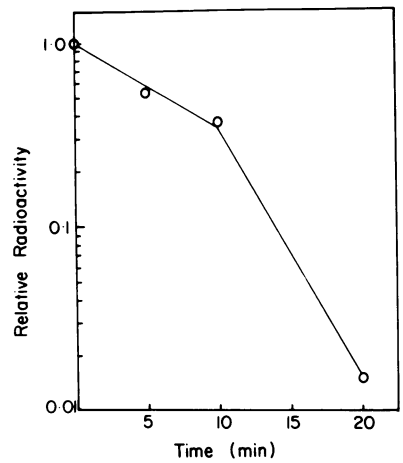

FIG. 4. *Effect of an increasing time period between UV irradiation of cells and infection by φ6 on the synthesis of viral protein P4. UV-irradiated Pseudomonas phaseolicola HB10Y was infected with φ6 at different times after irradiation. [^{14}C]leucine was added to the culture to a final concentration of 6 μCi/ml (at 10 min after infection), and the labeling continued until 85 min after infection. Samples were treated and electrophoresed as described in the text. The pattern of the autoradiograms was recorded with a Joyce-Loebl microdensitometer, and the relative amounts of P4 at various times were determined by estimating the areas under the individual peaks.*

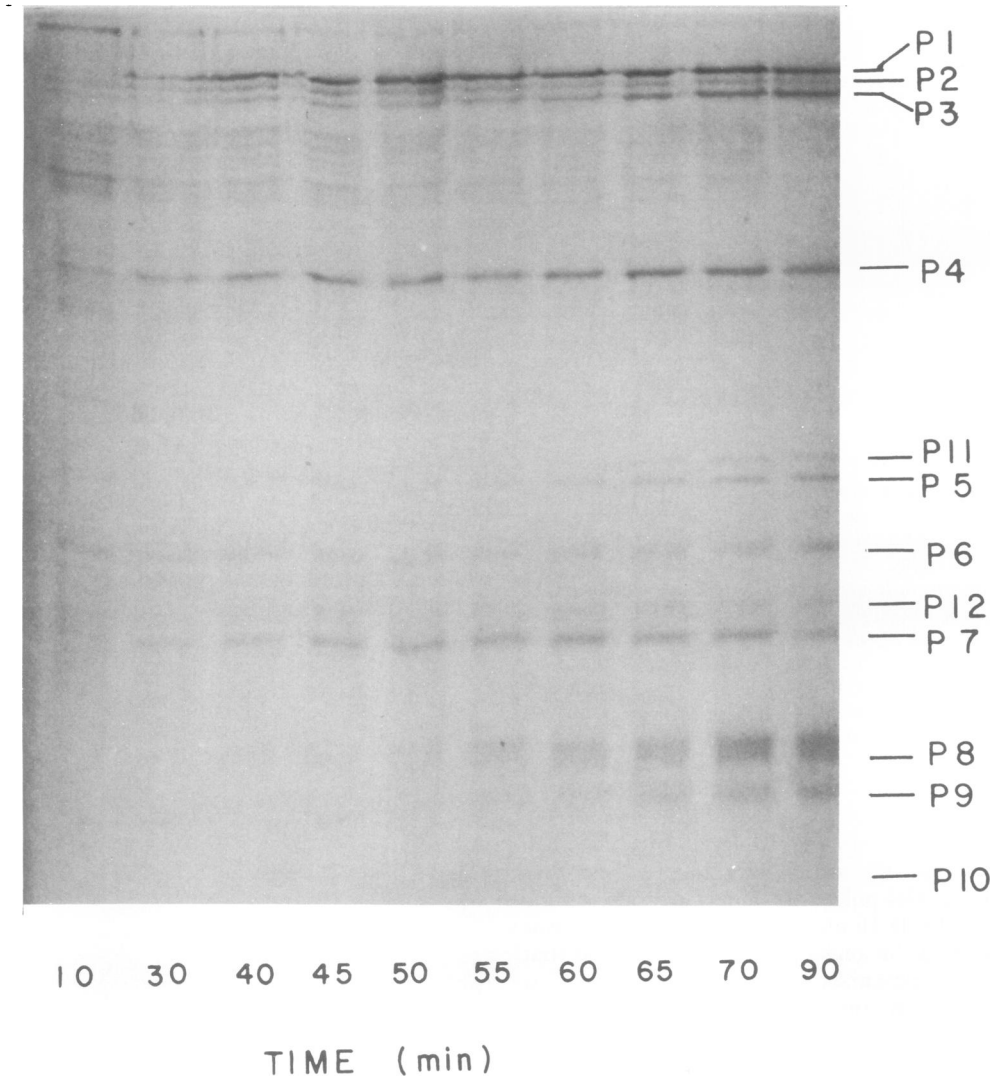

FIG. 5. *Kinetics of the appearance of φ6 proteins in extracts of infected cells. Cells were infected at MOI 50. At each time point indicated after infection, a sample from the infected cell culture was first treated with 50 μg of rifampin per ml for 10 min and then labeled with 2 μCi of leucine per ml for 2 min. The pulse was arrested by chilling the sample and immediately diluting it 1:5 into LC broth at 0 C. The conditions for electrophoresis and autoradiography are described in the text.*

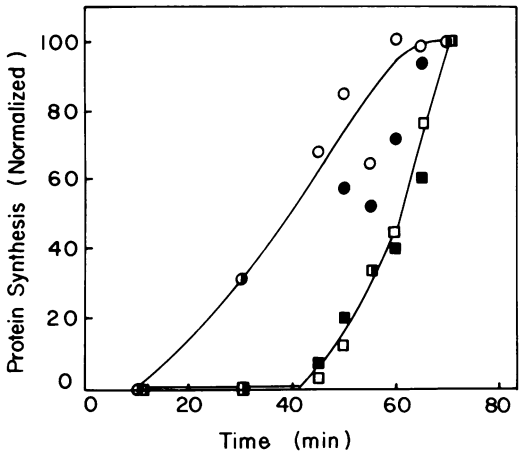

FIG. 6. *Appearance of viral proteins during infection. The extent of synthesis of four viral proteins at various times during infection was determined by analyzing the autoradiogram in Fig. 5 with a Joyce-Loebl microdensitometer. The results are presented for P4 (●), P7 (○), P8 (■), and P9 (□). The extent of synthesis at various times is normalized to that at 70 min.*

may be cleaved from a slightly larger polypeptide that is synthesized during infection.

From the order of appearance of viral proteins during infection (Fig. 5), it is evident that there is both an early and late class of viral proteins. This suggests that some type of regulation occurs during viral development. There is no turn-off of the synthesis of the early proteins since they are formed throughout the infection cycle and are found in the virion itself. This contrasts with the findings for T4 and T7 infecton (8, 10, 13, 14), in which synthesis of some early viral proteins is turned off later in infection. Several alternatives exist to explain the early-late control of protein synthesis. In T7 infection, the host RNA polymerase transcribes the early cistrons, among which is the gene for a new polymerase that is responsible for transcribing the rest of the phage genome (2). In the case of T4 infection, the regulation does not involve the synthesis of a new polymerase; instead, the host polymerase is modified during infection (10). Synthesis of φ6 mRNA may proceed in a manner similar to that of either T7 or T4. Alternatively, there may be a direct control of translation. The observation that UV treatment prevents late protein synthesis suggests that a host function is involved in the late processes. However, it is also possible that UV irradiation merely diminishes the synthesis of early proteins and therefore delays the appearance of the late proteins, so that in the time period observed (90 min) their synthesis has not yet begun. The finding that protein synthesis late in infection is sensitive to rifampin indicates that either a component of the host RNA polymerase is being used for late transcription or that the new polymerase is also rifampin sensitive. The sensitivity of the early protein synthesis to UV indicates that some newly synthesized host factor is involved in early transcription or translation. This could be a component of the host transcriptase, as is found for T4 and T7. Van Etten and co-workers have reported an RNA polymerase activity in purified virions (15). This enzyme could be involved in either early or late transcription or in the formation of the double-stranded RNA. These investigators have also claimed that phage-directed RNA synthesis occurs in the presence of rifampin (D. L. Coplin, J. L. Van Etten, and A. K. Vidaver, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, V 66, p. 211); however, it is not clear whether the elongation or initiation reactions were being measured in those experiments.

There are strong analogies between the φ6 infection and that of reovirus. Both contain double-stranded RNA in the genome, although in reovirus the pieces seem to be monocistronic and in φ6 they must be polycistronic; both have polymerase activity in the virion and both have selective synthesis of RNA or proteins early in the infection (11, 12, 15, 17). However, in the case of reovirus it is clear that the virion polymerase is responsible for the synthesis of early messenger. This is not established for φ6. We have found that rifampin added at the same time as phage prevents the synthesis of even early phage proteins (Fig. 5).

Since φ6 virions contain a dense icosahedral core surrounded by a lipid-containing envelope, it would be interesting to know which structural proteins comprise the envelope. We approached this problem by treating pure virus with Triton X-100, a process that removes the lipid envelope, as shown in the electron micrograph in Fig. 7. Proteins P3, P9, and P10 are completely extracted from the virus by this procedure (Fig. 8 and Table 1), and P6 is partially extracted; however, we cannot necessarily conclude that all of these proteins are on the periphery of the virion. It is possible that some internal proteins may be removed by Triton extraction due to an alteration in the structure of the virion. However, at least one, if not all four of the Triton-extractable proteins, is a possible candidate for

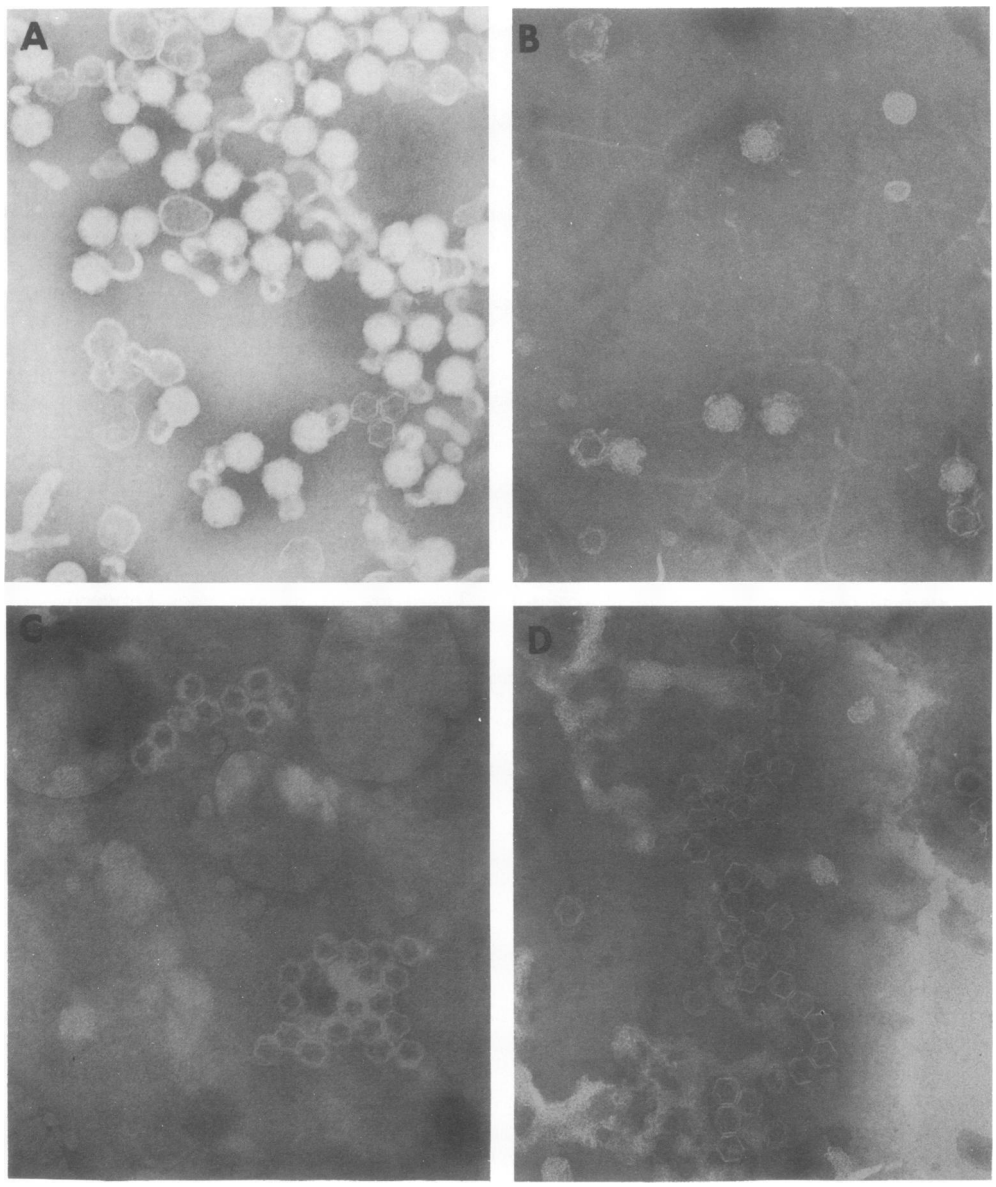

FIG. 7. *Effect of Triton X-100 on φ6 virions. Samples were suspended in 5 mM Tris-acetate buffer, pH 7.5, and treated with varying concentrations of Triton X-100. (A) Untreated; (B) 0.5%; (C) 1%; (D) 2%. Phosphotungstic acid stain. ×88,000.*

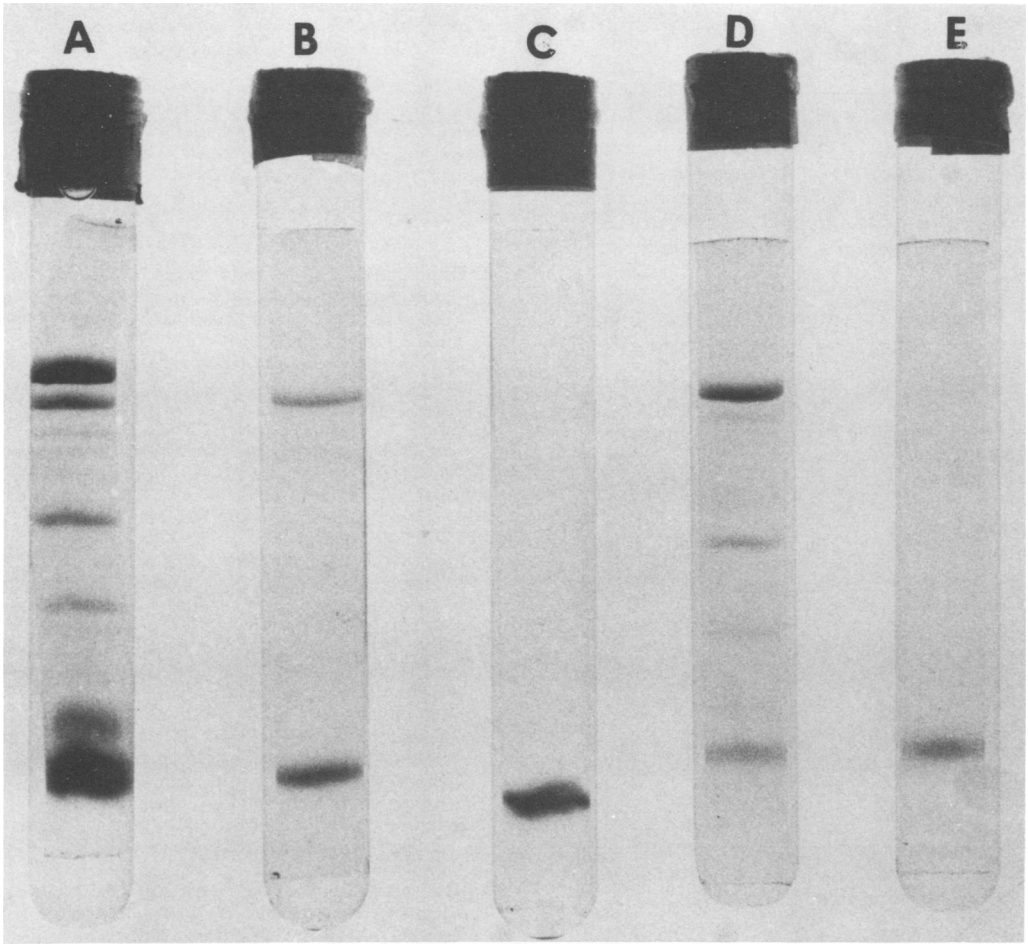

FIG. 8. *Extraction and isolation of viral proteins. Virions were extracted as described in the text and run in gels (18) and stained with amido black. (A) Pure virus; (B) material extracted with 1% Triton X-100; (C) protein P9 purified as in Fig. 8; (D) residue after Triton X-100 extraction; (E) protein P8 purified as in Fig. 9.*

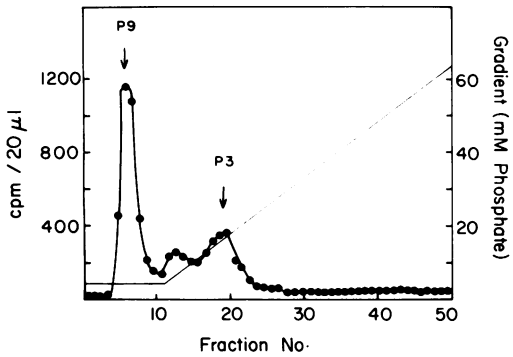

FIG. 9. *Purification of protein P9. Material extracted for φ6 with 1% Triton X-100 was applied to a column of DE-52 equilibrated in 0.2% Triton X-100, 5 mM sodium phosphate, pH 7.5, as described in the text.*

TABLE 2. *Amino acid composition of P9*[a]

Amino acid	Mol %	To nearest integer
Lysine	4.02	3
Histidine	1.22	1
Arginine	5.93	5
Aspartic acid	6.22	5
Threonine	5.53	5
Serine	5.34	4
Glutamic acid	10.52	9
Proline	5.65	5
Glycine	9.04	7
Alanine	14.51	12
Valine	9.33	8
Methionine	0	0
Isoleucine	6.50	5
Leucine	9.17	8
Tyrosine	1.51	1
Phenylalanine	5.56	5

[a] Protein P9 was isolated and analyzed as described in the text. Minimum mol wt = 8,728.

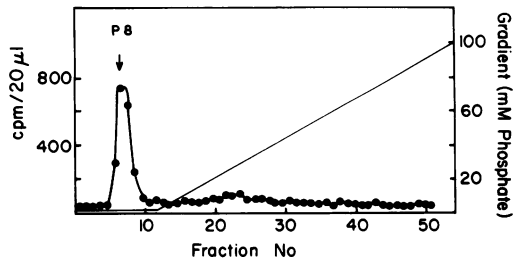

FIG. 10. *Purification of protein P8. The residue after Triton X-100 extraction was extracted with 6 M guanidine hydrochloride and applied to a column of DE-52 equilibrated in 8 M urea, 2 mM sodium phosphate, pH 7.5, as described in the text.*

the protein component(s) of the viral outer envelope. It is of interest that none of the "early" proteins are extractable with Triton, indicating that the outer envelope proteins are synthesized late.

Further investigations are being undertaken with mutants of φ6 to clarify the role of each viral protein in the development of the virion.

ACKNOWLEDGMENTS

We thank George Zulch for able technical assistance.

This investigation was supported in part by Public Health Service grants AI-09861 (from the National Institute of Allergy and Infectious Diseases) and GM-70474 (from the National Institute of General Medical Sciences) awarded to L.M.

LITERATURE CITED

1. Camerini-Otero, R. D., and R. M. Franklin. 1972. Structure and synthesis of a lipid-containing bacteriophage. XII. The fatty acids and lipid content of bacteriophage PM2. Virology **49**:385-393.
2. Chamberlain, M., J. McGrath, and L. Waskell. 1970. New RNA polymerase from *Escherichia coli* infected with bacteriophage T7. Nature (London) **228**:227-231.
3. Ellis, L. F., and R. A. Schlegel. 1974. Electron microscopy of *Pseudomonas* φ6 bacteriophage. J. Virol. **14**:1547-1551.
4. Espejo, R. T., and E. S. Canelo. 1968. Properties of bacteriophage PM2: a lipid containing bacterial virus. Virology **34**:738-747.
5. Espejo, R. T., E. S. Canelo, and R. L. Sinsheimer. 1969. DNA of bacteriophage PM2: a closed circular double-stranded molecule. Proc. Natl. Acad. Sci. U.S.A. **63**:1164-1168.
6. Grinsted, J. J., R. Saunders, L. C. Ingram, R. B. Sykes, and M. H. Richmond. 1972. Properties of an R factor which originated in *Pseudomonas aeruginosa* 1822. J. Bacteriol. **110**:529-537.
7. Hinnen, R., R. Schäfer, and R. M. Franklin. 1974. Structure and synthesis of a lipid-containing bacteriophage: preparation of virus and localization of the structural proteins. Eur. J. Biochem. **50**:1-14.
8. Hosoda, J., and C. Levinthal. 1968. Protein synthesis by *Escherichia coli* infected with bacteriophage T4D. Virology **34**:709-727.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
10. O'Farrell, P. Z., and L. M. Gold. 1973. Bacteriophage T4 gene expression: evidence for two classes of prereplicative cistrons. J. Biol. Chem. **248**:5502-5511.
11. Semancik, J. S., A. K. Vidaver, and J. L. Van Etten. 1973. Characterization of a segmented double-helical RNA from bacteriophage φ6. J. Mol. Biol. **78**:617-625.
12. Shatkin, A. J. 1971. Viruses with segmented ribonucleic acid genomes: multiplication of influenza versus reovirus. Bacteriol. Rev. **35**:250-266.
13. Studier, F. W. 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol. **79**:237-248.
14. Studier, F. W., and J. V. Maizel. 1969. T7-directed protein synthesis. Virology **39**:575-586.
15. Van Etten, J. L., J. K. Vidaver, R. K. Koski, and J. S. Semancik. 1973. RNA polymerase activity associated with bacteriophage φ6. J. Virol. **12**:464-471.
16. Vidaver, A. K., R. K. Koski, and J. L. Van Etten. 1973. Bacteriophage φ6: a lipid-containing virus of *Pseudom-*

onas phaseolicola. J. Virol. **11**:799-805.
17. Watanabe, Y., S. Millward, and A. F. Graham. 1968. Regulation of transcription of the reovirus genome. J. Mol. Biol. **36**:107-123.
18. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244**:4406-4412.