JOURNAL OF VIROLOGY, Feb. 1988, p. 400–406
0022-538X/88/020400-07$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 62, No. 2

Isolation of Bacteriophage T4 Baseplate Proteins P7 and P8 and In Vitro Formation of the P10/P7/P8 Assembly Intermediate

MARY F. PLISHKER,* SHAUKAT H. RANGWALA,† AND PETER B. BERGET‡

Department of Biochemistry and Molecular Biology, University of Texas Medical School and Graduate School of Biomedical Sciences, Houston, Texas 77225

Received 21 May 1987/Accepted 25 October 1987

Two bacteriophage T4 proteins, P7 and P8, which are components of the phage baseplate have been purified to apparent homogeneity. P7 and P8 are the protein products of T4 genes *7* and *8*. A plasmid has been constructed which contains approximately 5 kilobases of T4 DNA, including genes 7 and 8, under the control of the *tac* promoter. Induction of *Escherichia coli* W3110i^{Q} cells containing this plasmid resulted in the production of functional P7 and P8. Standard protein isolation procedures were used to purify both P7 and P8 from extracts of induced cells. In T4-infected cells, these two proteins and P10 interact in a strictly ordered sequential manner (P10 + P7 → P10/P7, P10/P7 + P8 → P10/P7/P8) to form an intermediate in the baseplate assembly pathway. The three purified proteins assembled in vitro to form a limited number of oligomeric species, as determined by nondenaturing gel electrophoresis. P10 and P7 interacted in vitro to form two assemblies with distinct electrophoretic mobilities, both containing P10 and P7. Addition of P8 to this mixture resulted in the disappearance of both P10/P7 species and the appearance of a single new assembly with a different electrophoretic mobility. These interactions occurred without the addition of any catalyst or cofactors. Isolated P11 appeared to add as predicted to the in vitro-formed complexes without affecting the formation of the two P10/P7 or the single P10/P7/P8 intermediates. Interactions between P7 and P8 in the absence of P10 or interactions between P10 and P8 in the absence of P7 could not be detected. These data indicate that purified P10, P7, and P8 interact in vitro in a manner completely in accord with the published assembly pathway and thus establish a system for further study of the regulation of the formation of this assembly intermediate in vitro.

The baseplate of bacteriophage T4 is the hexagonal organelle at the tip of the tail tube in the mature phage particle. This organelle functions during infection in the adsorption of the phage to the host cell and in the transfer of DNA from the phage head to the cell cytoplasm. The baseplate is assembled from two subassemblies, the wedge and the central hub (4–6). Six wedge-shaped structures assemble around a single hub to form the baseplate. Each wedge is the product of a linear ordered pathway of interactions of seven essential structural proteins (gp11, gp10, gp7, gp8, gp6, gp53, and gp25) and dihydrofolate reductase (gpfrd) (8) (see Materials and Methods for explanation of nomenclature). This pathway of assembly appears to be regulated by protein-protein interactions, each protein only adding to the growing structure when the required previous protein has been incorporated.

One approach to the study of the regulation of these interactions is to isolate unassembled component proteins and assemble them in vitro. Our previous studies of this pathway have included isolation and characterization of the component proteins of an intermediate in the assembly pathway, the P10/P11 complex. The two purified unassembled component proteins P10 and P11 do interact in vitro, as determined by nondenaturing polyacrylamide gel electrophoresis (PAGE), to form a functional P10/P11 intermediate (13). The carboxyl portion of gp11 is apparently important in this interaction (12). This study involves the in vitro assembly of the first strictly ordered intermediate in this pathway, the P10/P7/P8 complex. Factors which regulate the formation of this assembly intermediate require that the productive protein interactions occur in the order P10 + P7 → P10/P7, P10/P7 + P8 → P10/P7/P8. Assemblies containing only P7 and P8 or P10 and P8 have not been detected in vivo (4). The purpose of this study was to isolate the unassembled component proteins P7 and P8 and, together with previously isolated P10, examine the formation of the P10/P7 and P10/P7/P8 intermediates in vitro.

* Corresponding author.
† Present address: Monsanto Co., Chesterfield, MO 63198.
‡ Present Address: Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213.

MATERIALS AND METHODS

Buffers and media. L-broth contains (per liter) 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, and 1 ml of 1 M NaOH. Buffer A contains 10 mM Tris (pH 7.5), 1 mM EDTA, and 1 mM 2-mercaptoethanol. Buffer B contains 50 mM Tris (pH 7.5), 1 mM EDTA, 1 mM 2-mercaptoethanol, and 0.1 M NaCl. Buffer C contains 10 mM sodium phosphate, pH 7.5, 10% (vol/vol) glycerol, and 2 mM 2-mercaptoethanol. Isopropyl-β-D-thiogalactoside (IPTG) stock solutions (100 mM) were prepared in water and stored at −20°C. TBS contained 10 mM Tris, pH 7.6, and 0.125 M NaCl.

PAGE. Polyacrylamide gels containing sodium dodecyl sulfate (SDS) were prepared and run as described by Laemmli (11). The samples in 1× SDS sample buffer (0.0625 M Tris, pH 6.8, 10% glycerol, 5% 2-mercaptoethanol, 2% SDS, and 0.002% bromophenol blue) were placed in boiling water for 2 min prior to loading unless otherwise indicated. Nondenaturing gels were prepared without SDS or mercaptoethanol as previously described (13). Gels were stained with Coomassie blue. Samples for two-dimensional electrophoresis were subjected to electrophoresis under nondenaturing conditions as described above. The lane containing the sample was cut as a strip and placed in 1× SDS sample buffer in a boiling water bath for 2 min. The strip was then placed on top of a layer of SDS stacking gel (on a standard

SDS gel), cemented in place with melted agarose (1% in 25 mM Tris and 192 mM glycine), and electrophoresed as above. Prestained molecular weight standards (Diversified Biotech) were heated in boiling water prior to loading on SDS gels. This mixture contained the following protein standards (M_r): myosin (200,000), β-galactosidase (116,000), phosphorylase B (95,500), bovine serum albumin (68,000), ovalbumin (43,000), and trypsin inhibitor (20,400).

Protein assays. Protein concentrations were determined with the BioRad protein assay dye kit. Samples were diluted to 20 μl with water, 1.0 ml of diluted dye reagent was added, and the absorbance at 595 nm was determined. Samples of bovine gamma globulin were used as standards.

In vitro complementation. Protein samples (10 to 50 μl) were added to frozen pellets of T4 amber mutant-infected cell extracts as described (13). After incubation at 30°C for 2 h, the mixtures were diluted 100-fold, and viable phage titers were determined.

Construction of plasmid pSR10. Plasmid pSR10 (Fig. 1) contains T4 DNA from the *Cla*I site at coordinate 82.00 to the *Eco*RI site at coordinate 86.55 on the T4 physical map (9) under the control of the inducible *tac* promoter (1) in plasmid pBR322. This plasmid was constructed in several steps, which are detailed below.

Cytosine-containing T4 phage was prepared from T4alc7 as described by Kutter and Snyder (10). DNA prepared from these T4dC particles was digested to completion with *Hin*dIII and *Bam*HI. This DNA was mixed with the purified large fragment of pBR322 generated by digestion with the same enzymes and ligated with T4 DNA ligase. This ligation mix was transformed into *E. coli* MM294, selecting for ampicillin resistance. Because the T4 genome contains only one *Bam*HI site (15), only two types of T4 clones were expected: one containing T4 DNA from the *Bam*HI site (coordinate 85.30) to the *Hin*dIII site at coordinate 91.30, and the other containing T4 DNA from the *Bam*HI site to the *Hin*dIII site at coordinate 80.30, which should contain part of gene *6*, most if not all of gene *7*, and maybe a part of gene *8*. The desired clone (80.30 to 85.30) was recovered from the transformation and designated pPB20. From experiments which will be described elsewhere (Berget and Rangwala, manuscript in preparation), the start point of gene *7* translation was determined to be 18 nucleotides towards the *Bam*HI site from the *Cla*I site at T4 coordinate 82.00. The *Cla*I-*Bam*HI fragment of T4 DNA was subcloned into the unique *Pvu*II site in the expression plasmid ptac12 (1) after filling in the *Cla*I and *Bam*HI ends with deoxynucleotide triphosphates and the Klenow fragment of *E. coli* DNA polymerase I. The plasmid with the former *Cla*I site nearest the *tac* promoter was recovered and designated pSR8.

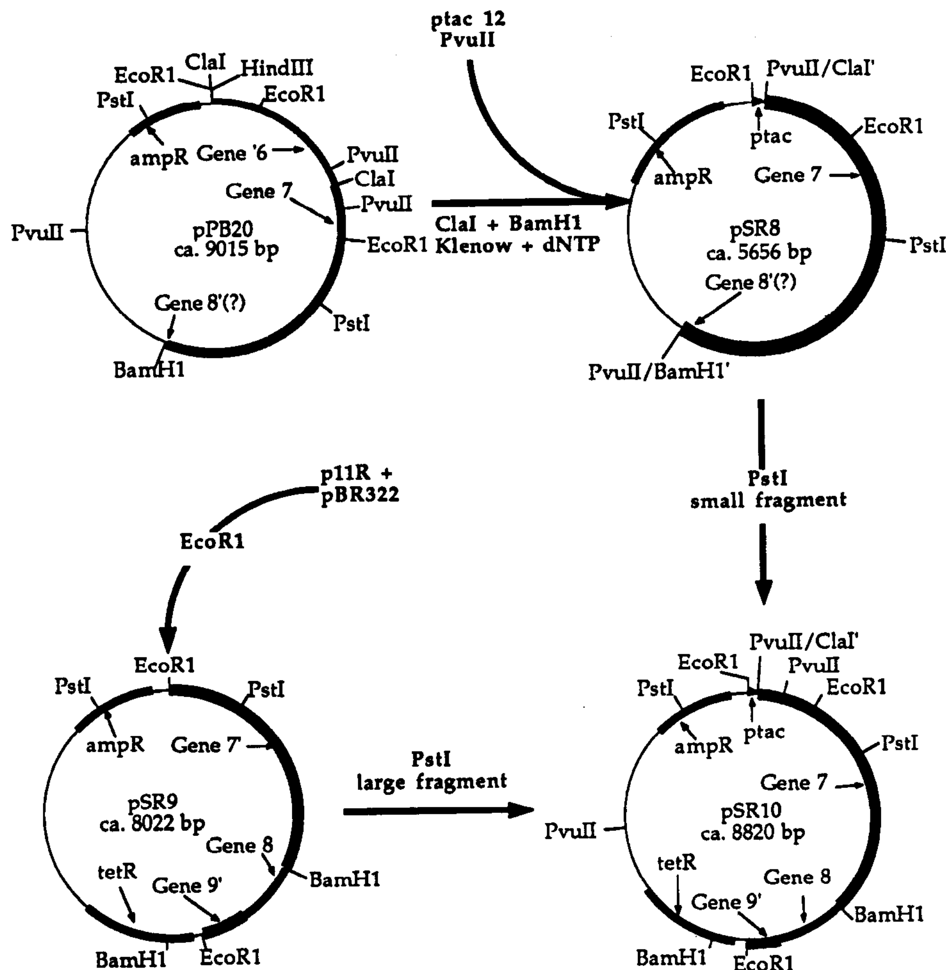

FIG. 1. Construction of plasmid pSR10. pSR10 was constructed from two *Pst*I fragments, each carrying T4 DNA, and is described in detail in the text. pSR9 is pBR322 carrying the T4 *Eco*RI fragment present in p11R (15). pPB20 contains a T4 *Hin*dIII-*Bam*HI fragment in a pBR322 backbone. pSR8 contains a T4 *Cla*I-*Bam*HI fragment under the transcriptional control of the *tac* promoter (1).

Plasmid p11R, provided by Geoff Wilson (15), carries the *Eco*RI fragment of the T4 genome from coordinates 82.70 to 86.55. This fragment was recloned into the *Eco*RI site of pBR322. The plasmid pSR9 carries this fragment with the *Eco*RI site at T4 coordinate 86.55 nearest the *Hin*dIII site of pBR322. Both pSR8 and pSR9 contain the *Pst*I site near T4 coordinate 83 and one other *Pst*I site in their respective beta-lactamase genes. In the final step to construct pSR10, the *Pst*I fragment containing the *tac* promoter from pSR8 was combined with the *Pst* fragment containing the *tet* (tetracycline resistance) gene from pSR9.

Purification of P7 and P8. All purification procedures were carried out at 4°C unless otherwise indicated. Cells containing pSR10 were grown at 37°C with aeration in 4 liters of L-broth to a density of 2×10^8 cells per ml. The culture was then induced by the addition of IPTG to a final concentration of 0.05 mM, followed by continued aeration at 37°C for 3 h. Cells were harvested by centrifugation (4,000 rpm, 10 min, Sorval GS-3). Cell pellets were saved and stored at −70°C (46 ml of cell slurry containing approximately 25 g of cells). Cells were opened by passage through a French pressure cell at 16,000 lb/in^2 into buffer A (final volume, 60 ml). The lysate was diluted and centrifuged for 60 min at 50,000 rpm (Beckman, Ti-50.2 rotor). The supernatant fraction (1.4 g of protein per 90 ml) was applied to a 30-ml column of DEAE resin equilibrated in buffer A. The column was washed with 250 ml of buffer A, followed by a 500-ml linear gradient of 0.1 to 0.4 M NaCl in buffer A. Fractions (6 ml) were collected and assayed for the presence of gp7 and gp8 by 7.5% SDS-PAGE and by in vitro complementation of $8^-/t^-$-infected cell extracts. Those fractions eluting between 0.15 and 0.27 M NaCl, containing both gp7 and gp8, were combined (210 mg/152 ml) and concentrated to 16 ml in an Amicon concentrator. The concentrated sample was chromatographed, as two 8-ml samples, on a BioGelA-0.5m column (2.5 by 85 cm) equilibrated in buffer B. Fractions (5 ml) were collected and assayed by 7.5% SDS-PAGE. Fractions containing gp7 and gp8 in overlapping peaks were combined (82 mg/170 ml) and applied to a 60-ml column of hydroxylapatite resin equilibrated in buffer C. The column was washed with 375 ml of buffer C, followed by a 1,000-ml linear phosphate gradient from 10 to 200 mM at the same pH. Fractions (7.5 ml) were collected and assayed by 7.5% SDS-PAGE. Fractions containing gp7 (5 mg/73 ml) and gp8 (40 mg/67 ml) were stored at −70°C.

Purification of P10 and P11. P10 was isolated from $11^-/7^-/t^-$-infected cells and P11 from $10^-/t^-$-infected cells as described previously (13). P10 (188,000 g/mol) was determined to be a dimer of gp10 (88,000) subunits: P10 = $gp10_2$. P11 (60,000 g/mol) was determined to be a dimer of gp11 (26,400 g/mol) subunits: P11 = $gp11_2$.

Preparation of antisera and serum-blocking assay. The anti-10, anti-7, and anti-8 sera were prepared by using P10 isolated as described previously (13) and P7 and P8 isolated as described above. A sample of P10 was denatured by incubation in 6 M guanidinium chloride, followed by dialysis to remove denaturant. Samples of native and denatured P10 (0.04 mg), P7 (0.07 mg), and P8 (0.28 mg) were injected into rabbits as described previously (13). Injection of antigens, bleeding, and serum production with P7 and P8 samples were performed by Bethyl Labs. The sera produced were analyzed by immunoblotting (described below) and in a serum-blocking assay described by Berget and King (2).

Immunoelectroblotting. Protein samples to be tested were adsorbed to nitrocellulose sheets by electrophoretic transfer from SDS-polyacrylamide gels or from nondenaturing gels as described by Towbin et al. (14). The nitrocellulose sheets were then soaked by shaking gently at 37°C in 50 ml of TBS containing 1% nonfat dry milk. A portion (50 to 100 μl) of appropriate antiserum was added to this, and the paper was soaked for 2 h at 37°C. After five washes with TBS, the nitrocellulose was soaked in 1% nonfat dry milk in TBS containing a 1/1,000 dilution of peroxidase-linked goat anti-rabbit immunoglobulin G (Sigma) for 2 h at 37°C. The sheet was washed extensively with TBS, and the bands were visualized with either 10 mM Tris, pH 7.5, containing 0.03% peroxide and *o*-dianisidine (25 mg/ml), or with 0.1 M Tris, pH 7.5, containing 0.03% peroxide, 0.2 M NaCl, and 4-chloro-1-naphthol (0.6 mg/ml). Staining was terminated by washing with water.

Nomenclature. The protein products of T4 genes which have been identified on SDS-PAGE and assigned to a particular gene are identified as gp (for gene product). Thus, gp8 indicates the protein product of gene *8*. The T4 proteins which have been purified as described below and which may exist in some native oligomeric structure are designated P. Thus, P8 is the protein isolated from W3110i^Q(pSR10) cells and identified on SDS-PAGE as gp8. The assembly formed from the interaction of P10, P7, and P8 is the P10/P7/P8 intermediate, and this intermediate contains gp10, gp7, and gp8.

RESULTS

Purification of active P7 and P8. The induction of cells containing pSR10 resulted in the increased production of proteins with mobilities similar to gp7 and gp8 as determined by SDS-PAGE analysis (Fig. 2, lane a). Addition of 50 μl of the lysates of these induced cells to in vitro complementation extracts defective in the production of gene *7* or *8* protein resulted in an increase of viable phage produced over background levels for both proteins (Fig. 3). The results of the SDS-PAGE analysis and the in vitro complementation assays together indicated that the induced plasmid-containing cells could be used as a source of these proteins.

A 4-liter culture of *E. coli* W3110i^Q(pSR10) cells was induced with IPTG, and the cell pellet was used as the starting material for isolation of P7 and P8. Samples taken at different steps in the purification procedures were subjected

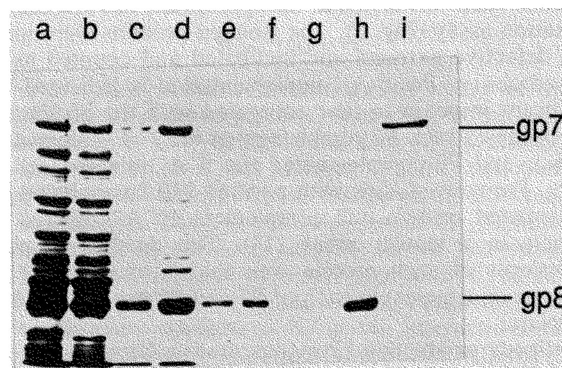

FIG. 2. Purification of P7 and P8. Samples were taken at each step in the purification of P7 and P8 from pSR10-containing cell extracts and subjected to electrophoresis on a 7.5% SDS-PAGE gel. Samples: a, crude extract; b, S100 supernatant; c, pooled fractions from DEAE column; d, concentrated sample from lane c; e, pooled sample from BioGel column; f, pool of P8 fractions from hydroxylapatite column; g, pool of P7 fractions from hydroxylapatite column; h and i, concentrated samples seen in lanes f and g, respectively.

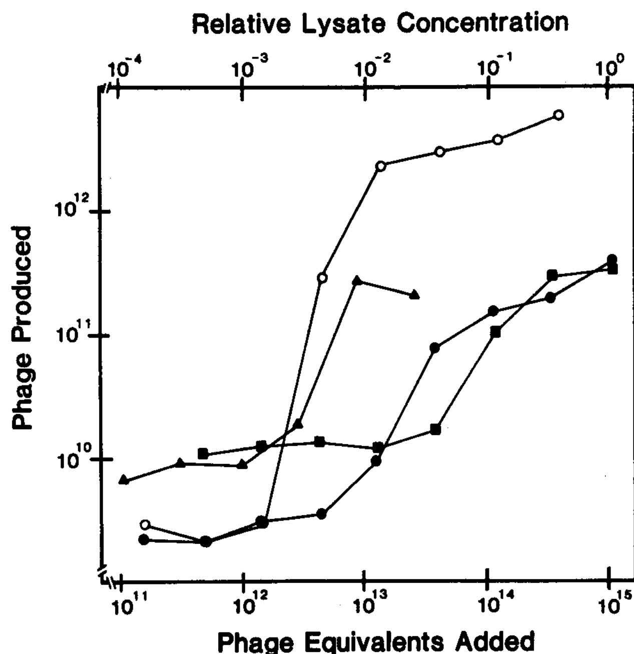

FIG. 3. In vitro complementation. Samples (50 μl) of serially diluted crude extracts of pSR10-containing cells were added to extracts of cells infected with $7^-/t^-$ (■) or $8^-/t^-$ (●) mutant phage, and phage were counted after 2 h of incubation as described in the text. Samples of purified P7 (▲) and P8 (○) were serially diluted and added to $7^-/t^-$ and $8^-/t^-$ extracts, respectively.

to SDS-PAGE analysis as shown in Fig. 2. The proteins coeluted from the DEAE column with several other contaminating proteins (lane d). The sample from the BioGelA-0.5m column (lane e) suggested that gp7 and gp8 coeluted from this column. There were actually two overlapping peaks (data not shown), which were combined for chromatography on the hydroxylapatite column. The two proteins eluted at distinct phosphate concentrations in the gradient applied to this column (Fig. 2, lanes f and g). Lanes h and i are concentrated samples of purified gp7 and gp8, each showing little, if any, contamination with other proteins. To determine whether the purified P7 and P8 were functional phage proteins, each was tested in the appropriate in vitro complementation assay (Fig. 3). The isolated proteins were mixed with defective extracts and incubated and counted as described above. Positive complementation is indicated as a significant increase in titer compared with the background titer of the extract. P7 complemented the $7^-/t^-$-infected cell extract, and P8 complemented the $8^-/t^-$-infected cell extracts. Previous studies with purified P10 have shown that this isolated protein will complement $10^-/t^-$-infected cell extracts in a similar assay (13). The number of phage equivalents of each protein was calculated based on the presence of 1 molecule each of P7 and P8 per wedge (6 per baseplate).

Antibody production. Previous studies have shown that antibodies generated against T4 proteins and assembly intermediates have been useful in purification of intermediates and component proteins (2, 3, 13). Antisera prepared against the three component proteins of the P10/P7/P8 intermediate would be useful in serum-blocking and immunoblot assays in the purification of any other assembly intermediates in which they are contained and in the characterization of intermediates formed from isolated proteins in vitro. To prepare these antisera, the purified unassembled proteins P10, P7, and P8 were injected into rabbits. The antiserum prepared with P10 differed from that previously described in that these antisera were prepared with P10 isolated from $11^-/7^-/t^-$-infected cells rather than with P10 from guanidine-denatured P10/P11 complex (2). Samples of native P10 (N10) and guanidine-denatured P10 (D10) were injected separately. The sera collected from all four injections were analyzed in serum-blocking assays and in immunoblots. Representative k values of the undiluted sera were: for anti-N10, 50 min^{-1}; for anti-D10, 150 min^{-1}; for anti-8, 0.6 min^{-1}; and for anti-7, 0.008 min^{-1}. Immunoblots with these antisera are described below (see Fig. 6).

In vitro assembly. The approach taken to follow the interaction of P10, P7, and P8 was based on the assay described previously (13) for the interaction of P10 and P11. Electrophoresis in a nondenaturing polyacrylamide gel system resulted in the separation of P10, P11, and the intermediate formed from these two proteins, the P10/P11 complex. The interactions followed in this study included the formation of an intermediate containing P10 and P7 (P10/P7) and, with the addition of P8, an intermediate containing P10, P7, and P8 (P10/P7/P8). Analysis of the formation of the P10/P7 intermediate in the nondenaturing PAGE system is shown in Fig. 4. P10 and P7 were mixed and incubated at room temperature for 20 min, and then the samples were subjected to electrophoresis. The mixtures of P10 and P7 displayed new bands with slower mobilities in this system (Fig. 4, lanes b to f and h to l). The mixing of P10 and P7 resulted in the disappearance of both the bands characteristic of P10 and P7 and the concomitant appearance of two new bands labeled P10/P7s (slow) and P10/P7f (fast) based on their relative mobilities in this PAGE system. An assembly of P10 and P7 as predicted previously (4) would contain 2 mol of gp10 and 1 mol of gp7: $gp10_2/gp7_1$, with a predicted molecular weight of 328,000 (based on a molecular weight of 140,000 for gp7). The mobility of such a complex would be expected to be slower than either P10 or P7; however, the appearance of more than one band was unexpected. Further examination of these species is described below.

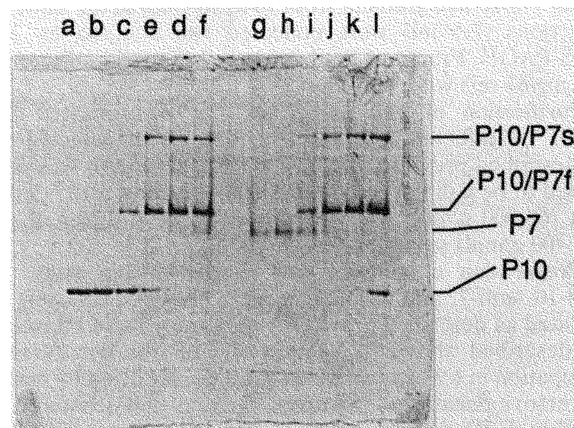

FIG. 4. In vitro assembly of P10 and P7. P10 (in buffer B containing 10% glycerol) and P7 (in buffer C) were mixed in a final volume of 40 μl as described below and incubated for 15 min at room temperature before electrophoresis in a 5.5% nondenaturing PAGE system. Lanes a to f each contained 2.6 μg of P10, to which were added the following amounts of P7: (a) none, (b) 0.2 μg, (c) 0.9 μg, (d) 1.8 μg, (e) 2.6 μg, or (f) 4.4 μg. Lanes g to l each contained 2.6 μg of P7, to which were added the following amounts of P10: (g) none, (h) 0.3 μg, (i) 1.4 μg, (j) 2.8 μg, (k) 4.2 μg, or (l) 7 μg.

The assembly containing P10, P7, and P8 was predicted to contain 2 mol of gp10 and 1 mol each of gp7 and gp8 ($gp10_2$/gp7/gp8) with a molecular weight of approximately 374,000 (based on a molecular weight of 46,000 for gp8). The addition of P8 to the mixture of P10 and P7 followed by electrophoresis resulted in the disappearance of both P10/P7 bands and the appearance of a new band of intermediate mobility (Fig. 5, lanes b to f and h to l). Whatever the differences between P10/P7f and P10/P7s, both appeared to be functional in terms of their ability to interact with P8, and the interactions resulted in the formation of a single electrophoretic species labeled P10/P7/P8. The assembly pathway as determined by Kikuchi and King (4) also predicts that mixtures of P10 and P8 or of P7 and P8 should not result in protein assembly. When these combinations were examined by the nondenaturing PAGE assay, the mixtures of P7 and P8 (lane p) and P10 and P8 (lane r) did not result in the appearance of new species or the disappearance of individual protein bands. The isolated proteins appeared to assemble in the order predicted.

The protein P11 is the exception to the rule in this ordered pathway and would not be expected to affect the binding of other proteins in the pathway to P10 (see Discussion). Figure 6 shows a 5.5% native gel and the banding patterns of assembly reactions in the presence of P11. There were two bands in the mixture of P10, P7, and P11 (lane e), labeled P11/P10/P7f and P11/P10/P7s, and a single band in the mixture containing P10, P7, P8, and P11 (lane g), labeled P11/P10/P7/P8. The addition of P11 appeared to shift the mobility of the structures to a slower position, but the general pattern appeared to be unchanged. This is further evidence that the proteins are behaving in vitro as has been predicted for the in vivo assembly.

Transfer of assembly mixtures from the nondenaturing gels to nitrocellulose and analysis in immunoblot assays showed binding of anti-P10 and anti-P7 sera to both P10/P7f and P10/P7s and binding of anti-P10, anti-P7, and anti-P8 sera to P10/P7/P8 (data not shown). There was unexplained cross-reactivity between both the anti-P8 and anti-P7 sera and P7 and P8; however, the immunoblot results supported the predicted compositions of the assembly intermediates.

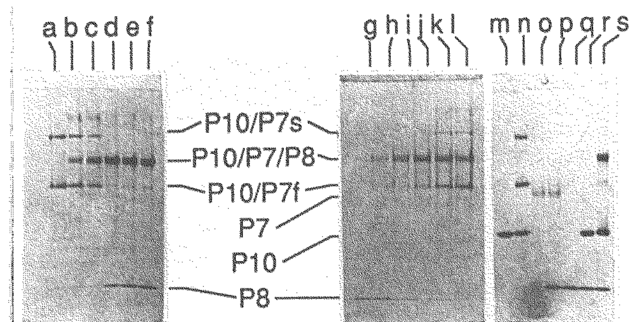

FIG. 5. In vitro assembly of P10, P7, and P8. P10 (2.8 μg) and P7 (1.8 μg) were incubated together as described in the legend to Fig. 4. This in vitro-formed P10/P7 complex was mixed with P8 and incubated for 15 min at room temperature. Lanes a to f each contained 4.6 μg of in vitro-formed P10/P7 complex, to which were added the following amounts of P8 (in a final volume of 30 μl): (a) none, (b) 0.2 μg, (c) 0.5 μg, (d) 1.2 μg, (e) 1.7 μg, or (f) 2.4 μg. Lanes g to l each contained 1.2 μg of P8, to which were added the following amounts of in vitro-formed P10/P7 complex (in a final volume of 34 μl): (f) none, (h) 0.5 μg, (i) 2.3 μg, (j) 4.6 μg, (k) 6.9 μg, or (l) 9.2 μg. Lanes m to s contained P10 (5 μg), P7 (2 μg), or P8 (3 μg) or the indicated mixtures of these amounts of the proteins: (m) P10, (n) P10 + P7, (o) P7, (p) P7 + P8, (q) P8, (r) P10 + P8, or (s) P10 + P7 + P8.

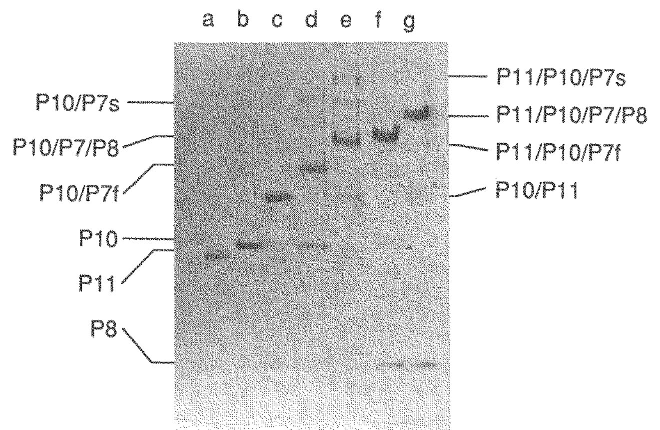

FIG. 6. Addition of P11 to in vitro assembly mixtures. The mixtures described below were incubated for 30 min at room temperature and then subjected to electrophoresis in a 5.5% nondenaturing PAGE system: (a) P11, (b) P10, (c) P11 + P10, (d) P10 + P7, (e) P11 + P10 + P7, (f) P10 + P7 + P8, (g) P11 + P10 + P7 + P8.

To determine the composition of the in vitro-formed intermediates P10/P7f, P10/P7s, and P10/P7/P8, the samples were subjected to electrophoresis in two dimensions, nondenaturing PAGE followed by SDS-PAGE (Fig. 7). Both P10/P7 bands contained gp10 and gp7 (Fig. 7A), and the P10/P7/P8 contained gp10, gp7, and gp8 (Fig. 7B). Neither sample appeared to contain any additional proteins. The ratio of spot densities of gp10 to gp7 in both P10/P7f and P10/P7s was 1.0:1.0. This molar ratio, based on staining, would be 1.6 to 1.0, in close agreement with a predicted composition of $gp10_2$/gp7 (4). These results are also consistent with conformational differences or dimerization of P10/P7 as the explanation for the appearance of two P10/P7 bands. The ratio of staining in Fig. 7B was 1.0:1.0:1.2 (gp10:gp7:gp8). The P10/P7/P8 band appeared to contain a complex with a composition of $gp10_2$/gp7/$gp8_3$ (1.6:1.0:2.6). The previous estimate of this composition, based on scans of stained gels and radioactivity, was $gp10_2$/gp7/gp8 (4). The reason for the presence of additional gp8 in this complex is not yet known. These results may reflect an artifact of protein staining.

DISCUSSION

The assembly of the one-sixth arm or wedge of the T4 baseplate is an ordered process, according to the pathway established in detail by Kikuchi and King (4) and Kozloff (8). The ordered nature of this pathway requires that there be precise controls to direct the formation of the macromolecular assemblies. There is no evidence in the literature to support temporal synthesis of different T4 late structural proteins, sequestering of subunits, or proteolysis as mechanisms of control of the baseplate assembly pathway. Control of this pathway appears to lie in the interactions of the component proteins. Interactions of subunits with the growing structure result in the formation or activation of sites to which subsequent subunits can bind. Soluble subunits are thus switched from unreactive to reactive states or switch previously assembled subunits when they bind in turn to the growing structure. Our approach to try to determine the changes occurring during this process, and during the formation of the sites for binding of succeeding proteins in the pathway, has been to isolate unassembled component pro-

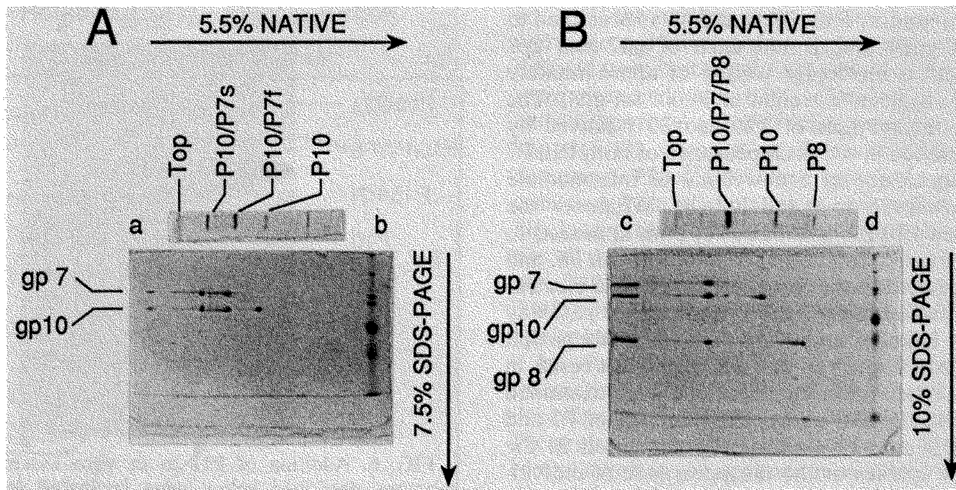

FIG. 7. Two-dimensional PAGE. Samples of P10 + P7 and P10 + P7 + P8 were incubated for 20 min at room temperature and then subjected to electrophoresis in a 5.5% native PAGE system. Slices of these gels were treated as described in the text, and electrophoresis in the second dimension was done on either a 7.5% (A) or 10% (B) SDS-PAGE system. A sample containing P10 (5.6 μg) and P7 (6 μg) was run on gel A, and a sample containing P10 (5.6 μg) and P7 (6 μg) and P8 (5.5 μg) was run on gel B. Lane a, P10 + P7 boiled in SDS sample buffer and run only in the second dimension; lane c, P10 + P7 + P8 treated in a similar fashion. Lanes b and d, Prestained SDS-PAGE molecular weight standards (see text) run only in the second dimension.

teins and follow their assembly in vitro. This has been accomplished for the first intermediate in this pathway, the P10/P11 complex (13).

The first strictly ordered intermediate in the 1/6th arm assembly pathway contains the protein products of genes *10*, *7*, and *8*, referred to as P10, P7, and P8, respectively. P10 and P7 combine in vivo to form an intermediate which contains an activated site to which P8 can then bind. Based on previous studies, it is predicted that this P10/P7/P8 intermediate contains a dimer of gp10 and monomers of gp7 and gp8 (4). This is the system which we have begun to study as described here.

P10 was isolated as previously described from cells infected with $11^-/7^-/t^-$ mutant T4 phage (13). This method of production of unassembled protein was successful but required 300 g of infected cells for isolation of 7.5 mg of phage protein. We have described here the construction of a plasmid, pSR10, which contains T4 DNA encoding both genes *7* and *8*. The production of these two proteins is under the control of an inducible promoter. Proteins produced in response to induction of plasmid-containing cells correspond to P7 and P8 when cell extracts are analyzed by SDS-PAGE and by in vitro complementation assays. Both proteins are apparently produced by these cells in an active form. The extract from these cells was subjected to standard protein purification treatments to isolate P7 and P8. Column chromatography on DEAE, BioGelA-0.5m, and hydroxylapatite resins resulted in the isolation of 5.2 mg of pure P7 and 39 mg of pure P8 from a 4-liter culture of induced cells. The purified proteins were also active in the in vitro complementation assays (Fig. 3).

All three purified proteins, P7, P8, and P10, were used to produce antisera. Anti-10, anti-7, and anti-8 sera inactivated T4 phage, anti-10 being the most efficient. This may be the result of greater accessibility of P10 due to its location at the vertices of the hexagonal baseplate in the assembled phage particle (2). The results of the serum-blocking assays with purified proteins used to block the sera demonstrate the specificity of these antisera. Anti-10 was blocked by purified P10 (data not shown). Anti-10 was blocked less than 30% by 10^--infected cell extracts (containing all other phage proteins). Anti-7 and anti-8 sera cross-reacted to a limited extent. Both sera bound to both P7 and P8 in immunoelectroblot assays. In addition, anti-7 was at least partially blocked by both P7 and P8 in serum-blocking assays. This cross-reactivity may be the result of cross-contamination of protein samples which were injected or of antigenic similarities in P7 and P8. Though we have not yet determined the reason for this cross-reactivity, the sera are still useful in protein and assembly intermediate isolation and in some of the in vitro assembly analyses, since the three proteins display characteristic mobilities in both SDS and nondenaturing PAGE systems.

The nondenaturing polyacrylamide gel system used to examine the interaction of T4 proteins P10 and P11 (13) was also found to be applicable to the interactions of P10, P7, and P8. The mixing of P10 and P7 resulted in the disappearance of both the P10 and P7 bands in this PAGE system, concomitant with the appearance of two new bands, designated P10/P7f and P10/P7s (Fig. 4). Both new bands appeared to contain P10 and P7, based on two-dimensional PAGE (Fig. 7) and supported by immunoelectroblotting (data not shown). Addition of P8 to the in vitro-assembled P10 and P7 resulted in the disappearance of both P10/P7 bands and the appearance of a single band of intermediate mobility (Fig. 5). Two-dimensional electrophoresis (Fig. 7) indicated that P10, P7, and P8 were all present in this new electrophoretic species.

The compositions of the three intermediates based on scans of the second-dimension SDS-PAGE gels were estimated to be $gp10_2/gp7$ for both P10/P7f and P10/P7s and $gp10_2/gp7/gp8_3$ for P10/P7/P8. The predicted composition of P10/P7/P8 is $gp10_2/gp7/gp8$, based on gel staining and radioactivity (4). P10 and P7 appear to be present in predicted amounts both in P10/P7 bands and in P10/P7/P8. Whether the presence of additional P8 represents an artifact of protein staining of in vitro assembly or electrophoresis has not yet been determined. Further analysis of P10/P7/P8 will include comparisons with in vivo-formed baseplate intermediates containing gp8 (P10/P7/P8, P10/P7/P8/P6, etc.) under similar electrophoretic conditions.

P11 is found in association with P10 in the absence of P7 (3, 4, 13), and formation of the P10/P11 intermediate is

included as a first step in the assembly pathway. This is, however, not an obligatory step. The baseplate can be assembled in the absence of P11. Under this condition (11^- mutant T4), gp12, which associates with wedges after radial polymerization, cannot add to the structure (7). Thus, the addition of P11 to in vitro assembly mixtures of baseplate proteins P10, P7, and P8 would not be expected to affect the observed interactions. As shown above (Fig. 6), P11 apparently adds to the in vitro-formed intermediates, shifting the mobilities to lower values. There appears to be no effect on the formation of the two P10/P7 species, since the number of bands was unchanged; only the mobilities were shifted.

The two P10/P7 bands could represent different stoichiometric forms of the P10/P7 intermediate; that is, one form could be $gp10_2/gp7$ and the other $gp10_4/gp7_2$. Addition of P8 may prevent the association of P10/P7 either by steric hindrance or by causing conformational changes. Attempts were made to separate P10/P7f from P10/P7s by possible molecular weight differences. Chromatography on BioGelA-0.5m and BioGelA-1.5m resulted in the coelution of P10/P7f and P10/P7s when fractions were assayed by nondenaturing PAGE. It is also possible that the two bands of P10/P7 seen on nondenaturing gels could represent two conformations of the P10/P7 complex, perhaps involved in the regulation of P8 addition, which are somehow resolved by this separation technique. Examination of $11^-/8^-$-infected cell extracts for the presence of these species should indicate whether the assemblies represented by either or both of these bands exist during the in vivo assembly process.

The data presented here support the idea that the controls for the assembly of the T4 baseplate reside in the component structural proteins and are present in the purified proteins. The four isolated, unassembled functional proteins apparently interact in vitro, providing us with a system with which to probe those aspects of protein structure which may be involved in regulation of the formation of assembly intermediates. Other components of this assembly pathway, including gp6, gp53, and gp25, may be similarly isolated and their interactions assayed.

ACKNOWLEDGMENTS

We acknowledge the technical assistance of Monjula Chidambaram, Roger Kylberg, Claire Whelan, Harryette Davis, Kevin Reidy, and Bill Black. We also thank Theresa Domany for help in the preparation of this manuscript.

This research was supported by Public Health Service grants GM-33532 (M.F.P.) and GM-28952 (P.B.B.) from the National Institutes of Health and by National Science Foundation grant PCM8314241 (P.B.B.).

LITERATURE CITED

1. **Amann, E., J. Brosius, and M. Ptashne.** 1983. Vectors bearing a hybrid trp-lac promoter useful for regulated expression of cloned genes in Escherichia coli. Gene **25**:167–178.
2. **Berget, P. B., and J. King.** 1978. Antigenic gene products of bacteriophage T4 baseplates. Virology **86**:4350–4354.
3. **Berget, P. B., and J. King.** 1978. The isolation and characterization of precursors in T4 baseplate assembly. The complex of gene 10 and gene 11 products. J. Mol. Biol. **124**:469–486.
4. **Kikuchi, Y., and J. King.** 1975. Genetic control of bacteriophage T4 baseplate morphogenesis. I. Sequential assembly of the major precursor, *in vivo* and *in vitro*. J. Mol. Biol. **99**:645–672.
5. **Kikuchi, Y., and J. King.** 1975. Genetic control of bacteriophage T4 baseplate morphogenesis. II. Mutants unable to form the central part of the baseplate. J. Mol. Biol. **99**:673–694.
6. **Kikuchi, Y., and J. King.** 1975. Genetic control of bacteriophage T4 baseplate morphogenesis. III. Formation of the central plug and overall assembly pathway. J. Mol. Biol. **99**:695–716.
7. **King, J., and N. Mykolajewycz.** 1973. Bacteriophage T4 tail assembly: proteins of the sheath, core and baseplate. J. Mol. Biol. **75**:339–358.
8. **Kozloff, L. M.** 1981. Composition of the T4D bacteriophage baseplate and the binding of the central tail plug, p. 327–342. *In* M. Dubow (ed.), Bacteriophage assembly. Alan R. Liss, New York.
9. **Kutter, E., and W. Rüger.** 1983. Map of the T4 genome and its transcriptional control sites, p. 277–290. *In* C. K. Mathews, E. M. Kutter, G. Mosig, and P. B. Berget (ed.), Bacteriophage T4. American Society for Microbiology, Washington, D.C.
10. **Kutter, E., and L. Snyder.** 1983. Production of cytosine-containing T4 phage, p. 56–57. *In* C. K. Mathews, E. M. Kutter, G. Mosig, and P. B. Berget (ed.), Bacteriophage T4. American Society for Microbiology, Washington, D.C.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Plishker, M. F., and P. B. Berget.** 1984. Isolation and characterization of precursors in T4 baseplate assembly. III. The carboxy-termini of the bacteriophage T4 baseplate protein P11 are required for assembly activity. J. Mol. Biol. **178**:699–709.
13. **Plishker, M. F., M. Chidambaram, and P. B. Berget.** 1983. Isolation and characterization of precursors in T4 baseplate assembly. II. Purification of the genes 10 and 11 precursors and *in vitro* assembly of the P(10/11) complex. J. Mol. Biol. **170**: 119–135.
14. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
15. **Wilson, G. G., R. L. Neve, G. J. Edlin, and W. N. Konigsberg.** 1980. BamHI restriction site in the bacteriophage T4 chromosome is located in or near gene 8. Genetics **93**:285–296.