

## Isolation and Characterization of Precursors in Bacteriophage T4 Baseplate Assembly

### III.† The Carboxyl Termini of Protein P11 are Required for Assembly Activity

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The assembly activity and electrophoretic mobility of a T4 bacteriophage baseplate protein, P11, have been found to be affected by digestion with the proteases trypsin, subtilisin and carboxypeptidase Y. Analysis of the trypsin limit-digestion product of P11 by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and size analysis by high performance liquid chromatography indicate that there is a decrease of approximately 5000 in the molecular weight of the P11 molecule or a loss of 2500 in  $M_r$  from each of the gp11 subunits of the dimer. During protease treatment P11 demonstrates a time-dependent loss in the ability to interact with the baseplate protein P10 to form the P(10/11) complex, the first assembly intermediate of the T4 baseplate 1/6th arm. Similar treatments of the P(10/11) complex indicate that P11 in the complex is not affected by these proteases. Concomitant with the loss of assembly activity is a change in the electrophoretic mobility of P11 on non-denaturing polyacrylamide gels from a single band to a series of more mobile bands suggesting sequential loss of positive charge. P11 assembly activity is completely lost after removal of the first positive charge. These results suggest that the carboxyl termini of the two gp11 subunits of the P11 molecule are involved in the interaction of P11 with P10 to form the P(10/11) complex. Analysis of the portion of gp11 removed by carboxypeptidase Y demonstrates that there are up to 13 aliphatic and aromatic carboxyl-terminal amino acids.

### 1. Introduction

The baseplate of bacteriophage T4 is the hexagonal adsorption organelle at the distal end of the phage tail. This substructure is involved in host-cell recognition and the delivery of phage chromosome into the cytoplasm. The baseplate is formed by the assembly of six wedge-shaped 1/6th arms around a central hub. Each of these 1/6th arms is assembled from eight phage proteins (gp10, gp11, gp7,

† Paper II in this series is by Plishker *et al.* (1983).

gp8, gp6, gpfrd, gp53 and gp25) in a linear series of protein interactions (Kikuchi & King, 1975*a,b,c*; Mosher & Mathews, 1979; Kozloff, 1981). Five of the seven assembly reactions in this pathway are controlled by the addition of the previous protein in the sequence without the intervention of any known catalytic factors. Thus 1/6th arm assembly is regulated by protein-protein interactions. This suggests that reactive assembly sites are created either by conformational changes in the assembly proteins, by the formation of assembly sites from partial sites on different protein molecules or by a combination of these two possibilities (Berget & King, 1983). This type of "heteroco-operative" interaction (Wood, 1980) has not been experimentally established at the molecular level.

The first intermediate in the assembly of the 1/6th arm is the P(10/11) complex, previously isolated and characterized by Berget & King (1978). The intermediate contains only the protein products of genes 10 and 11, gp10 and gp11. The native forms of the two precursor proteins, P10 and P11, have also been isolated and characterized (Plishker *et al.*, 1983). P10, a dimer of gp10, and P11, a dimer of gp11, interact *in vitro* to form only one oligomeric species. This *in vitro*-formed oligomer is identical to the *in vivo*-formed P(10/11) complex. Because these two proteins do assemble *in vitro* in a manner similar or identical to that occurring *in vivo*, and the assembly product is easily identified on non-denaturing gels, the examination of the interaction between P10 and P11 can now proceed on a molecular basis. In this paper we present evidence that suggests that the carboxyl terminus of gp11, in the P11 molecule, is the domain that interacts with P10 during assembly.

## 2. Materials and Methods

### (a) Chemicals

Trypsin, subtilisin, fluorescamine, dansyl chloride, dansyl amino acid standards and phenylmethylsulfonyl fluoride were obtained from Sigma Chemical Company. Carboxypeptidase Y powder, lyophilized from 0.1 sodium citrate, was obtained from Pierce Chemical Company. Trypsin was dissolved in 1 mM-HCl, subtilisin and carboxypeptidase Y were dissolved in water.

### (b) P10, P11 and P(10/11) complex

P10 and P11 were isolated as previously described (Plishker *et al.*, 1983). Unless otherwise indicated both proteins were in 50 mM-Tris·HCl (pH 7.5), 1 mM-2-mercaptoethanol, 0.1 M-NaCl. To assay for the assembly activity of P10 and P11, the 2 proteins were mixed and resolved by electrophoresis in 7.5% (w/v) non-denaturing polyacrylamide gels. The extent of assembly was monitored by the appearance of a band corresponding to the mobility of *in vivo*-assembled P(10/11) complex. Standard samples of *in vitro*-formed P(10/11) complex were prepared by incubation of equimolar amounts of P10 with P11 for 2 h at 37°C. *In vivo*-formed P(10/11) complex was isolated as previously described (Berget & King, 1978).

### (c) Proteolysis

To start the incubation, the protease was added to a solution of the purified structural protein. At specific times, samples were removed and the proteolysis was stopped by the

addition of PMSF† (to 1 mM). These samples were examined directly by sodium dodecyl sulfate and/or non-denaturing polyacrylamide gel electrophoresis. Additional samples from each time-point were assayed for assembly activity by the addition of the appropriate assembly protein and subsequent separation by electrophoresis on a non-denaturing gel. Limit digests are the result of 90 min of digestion (at the indicated protease concentration) at 37°C.

#### (d) *Electrophoresis*

The denaturing gels, 12.5% (w/v) polyacrylamide containing 0.1% SDS, were prepared as described by Laemmli (1970) and also contained 6 M-urea. Non-denaturing 7.5% polyacrylamide gels were identical to SDS-containing gels except that no SDS was included in the gel matrix and no mercaptoethanol or SDS was added to the sample buffer. Samples for non-denaturing gel analysis were not boiled before loading on the gel. Gels were stained with Coomassie Brilliant blue (0.13 mg/ml) in a solution of methanol/acetic acid/water (93 : 93 : 14, by vol.).

#### (e) *High performance liquid chromatography*

A 20 µg sample of P11 was treated with 0.3 µg trypsin for 90 min at 37°C. The proteolysis was stopped by the addition of PMSF. This sample was then subjected to chromatography on a Beckman TSK 3000 column equilibrated in 50 mM-Tris·HCl (pH 7.5), 0.1 M-NaCl using a Beckman HPLC system. Untreated P11, bovine serum albumin, ovalbumin, ribonuclease and thyroglobulin were used as molecular weight standards.

#### (f) *Isolation of tryptic peptides*

A sample of P11 (1.2 mg) was dialyzed into 50 mM-ammonium bicarbonate (pH 7.8). Trypsin (6.0 µg) in 1.0 mM-HCl was added and the mixture incubated at 37°C for 90 min. The proteolysis was stopped by the addition of PMSF. The mixture was concentrated by lyophilization, resuspended in 0.1 ml of 50 mM-ammonium bicarbonate and chromatographed on a Sephadex G-50 column (1.2 cm × 45 cm) equilibrated with the same buffer. Fractions containing the included peak of ultraviolet-absorbing material were combined and concentrated by lyophilization. These tryptic peptides were hydrolyzed for 24 h in 6 M-HCl at 110°C then analyzed on a Beckman model 119 amino acid analyzer.

#### (g) *Sequencing with carboxypeptidase Y*

Samples of P11 in 50 mM-Tris·HCl (pH 7.5), 1 mM-EDTA, were incubated with several concentrations of carboxypeptidase Y at 37°C. Portions of these digestion mixtures were removed at specific times and the proteolysis was stopped by the addition of HCl to pH 2. These samples were clarified by centrifugation and applied directly to the amino acid analyzer to determine which amino acids had been released.

#### (h) *In vitro complementation*

Samples of *in vivo*-formed P(10/11) complex and *in vitro*-formed P(10/11) complex (30 µg) were incubated in either the absence or the presence of trypsin (0.15 µg) for 90 min at 37°C. PMSF was added to stop proteolytic activity, and the samples were added to 10<sup>-11</sup>/t<sup>-</sup> extracts and analyzed as previously described (Plishker *et al.*, 1983).

† Abbreviations used: PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; HPLC, high performance liquid chromatography.

(i) *Amino-terminal amino acid determination*

Samples of P11 that had been treated with each protease (trypsin, subtilisin or carboxypeptidase Y) were chromatographed on Sephadex G-50 (as described above). The peak fraction of the excluded ultraviolet-absorbing peak of each digest was concentrated by lyophilization then resuspended in and dialyzed against 0.1 M-sodium bicarbonate. These "cores" of P11 were dansylated, hydrolyzed in 6 M-HCl at 110°C and analyzed on polyamide layer sheets (Woods & Wang, 1967; Weiner *et al.*, 1972).

### 3. Results

#### (a) *Rationale*

To determine domains of assembly function of the purified structural proteins P10 and P11 of the T4 baseplate, these proteins were treated with several proteases. The effects of these proteases were analyzed from several points of view. What was the effect of these proteases on the electrophoretic mobility of the structural proteins when examined by SDS and by non-denaturing polyacrylamide gel electrophoresis? Does the protease treatment have any effect on the assembly activity of P11 and P10 to form the P(10/11) complex? Does prior assembly of P11 and P10 change the accessibility of these proteins to the proteases? In answering these questions, subtilisin, trypsin and carboxypeptidase Y were found to be useful probes into the structure of P11 and its assembly into the P(10/11) complex.

#### (b) *Sensitivity of P10, P11 and P(10/11) to proteases*

Under all native conditions used, the T4 baseplate protein P10 showed no sensitivity to proteases by either physical analysis or biological assay (data not shown). However, incubation of P11 with the bacterial protease subtilisin results in a time-dependent change in the mobility of P11 on a non-denaturing gel, as shown in Figure 1. The P11 pattern changes from a single band to series of more mobile bands, which seem to appear and disappear sequentially (lanes a to f). Samples of the digestion reaction were removed at various times and the proteolysis was stopped by the addition of PMSF. Portions of these samples were assayed for P11 assembly activity by incubation with P10 (Plishker *et al.*, 1983). These assembly assays are shown in lanes g to k. The pattern of formation of the band corresponding to the P(10/11) complex indicates that there is a time-dependent loss in the ability of the P11 in these digestion reactions to assemble with P10. Assembly activity is completely lost when the native P11 band is no longer visible (lanes e and j). A sample of *in vitro*-formed P(10/11) complex, similarly treated (lane m), showed no change in mobility when compared with an untreated sample (lane l). Thus the disappearance of the P(10/11) band (lanes g to k) is not due to destruction of assembled complex by residual protease. Incubation of P11 with trypsin at a weight ratio of 1 : 200 (trypsin : P11) or with carboxypeptidase Y at a weight ratio of 1 : 25 (carboxypeptidase : P11) results in a similar time-dependent change in the native gel pattern and assembly activity of P11 (data not shown).

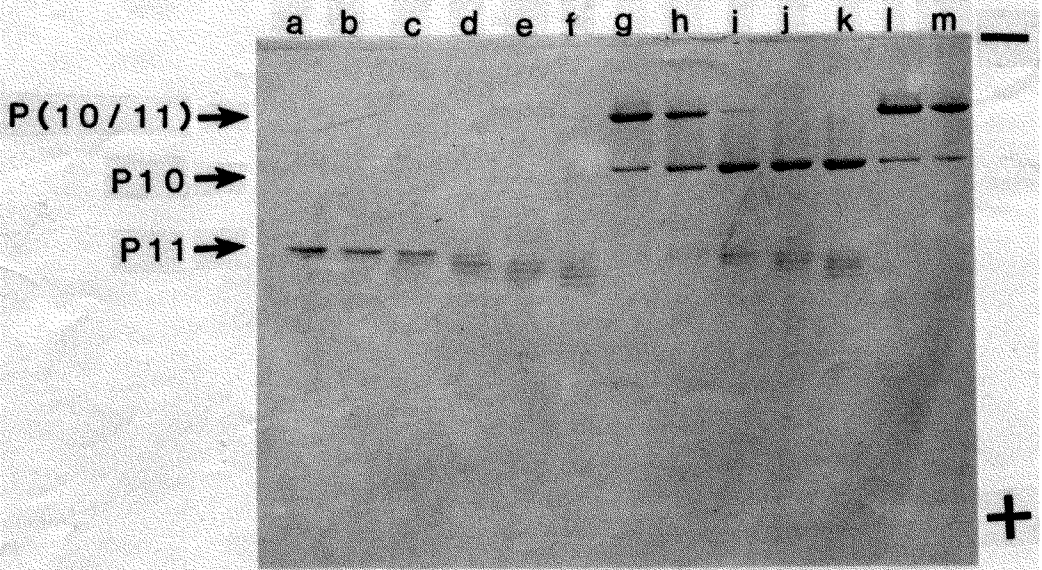


FIG. 1. Effect of subtilisin on P11. A 26- $\mu$ g sample of P11 in 0.175 ml of 50 mM-Tris  $\cdot$  HCl (pH 7.5), 1 mM-EDTA was incubated at 37°C with 80 ng subtilisin. After 1, 5, 15, 30 and 60 min of incubation, 28  $\mu$ l were removed from the mixture and PMSF was added to stop the digestion. A 14- $\mu$ l portion of the sample at each time-point was added to 5  $\mu$ l P10 (0.6 mg/ml). The digested P11 samples (lanes b to f) and mixtures of digested P11 and P10 (lanes g to k) were examined on a 7.5% non-denaturing polyacrylamide gel. A 5- $\mu$ g sample of *in vitro*-formed P(10/11) complex (19  $\mu$ l) was incubated with 15 ng subtilisin for 60 min at 37°C, the reaction was stopped with PMSF, and the sample was analyzed on the same gel (lane m). Untreated P11 (lane a) and P(10/11) (lane l) were run as controls. The digestion times for P11 are: 1 min (lanes b and g), 5 min (lanes c and h), 15 min (lanes d and i), 30 min (lanes e and j), 60 min (lanes f and k). The mobilities of P10, P11 and the P(10/11) complex in this electrophoretic system are indicated on the left.

Samples of P11, which had been incubated for 90 minutes with trypsin, subtilisin or carboxypeptidase Y (limit digests), were examined by 12.5% (w/v) polyacrylamide gel electrophoresis containing 0.1% SDS and 6 M-urea (Fig. 2, lanes a to d). Treatment with either trypsin (lane b), subtilisin (lane c) or carboxypeptidase Y (lane d) results in a small increase in the mobility of the gp11 subunit of P11. These proteases do not hydrolyze gp11 into a series of small peptides but apparently cleave the gp11 molecule near one end (the carboxyl terminus) of the polypeptide chain. Similar treatment of the P(10/11) complex formed *in vitro* (lanes e to h) or *in vivo* (lanes i to l) results in no detectable change in the mobility of either the gp11 or gp10 subunits of the complex. Samples of P(10/11) complex treated with trypsin were analyzed for their ability to be incorporated into functional phage particles by *in vitro* complementation as described in Materials and Methods. The level of complementation as described in Materials and Methods. The level of complementation of  $10^{-}/11^{-}$ -defective extracts by both *in vivo* and *in vitro*-formed P(10/11) samples was unchanged by prior incubation of the sample with trypsin (data not shown). The protease-sensitive sites on P11 are apparently inaccessible when P11 is in the assembled

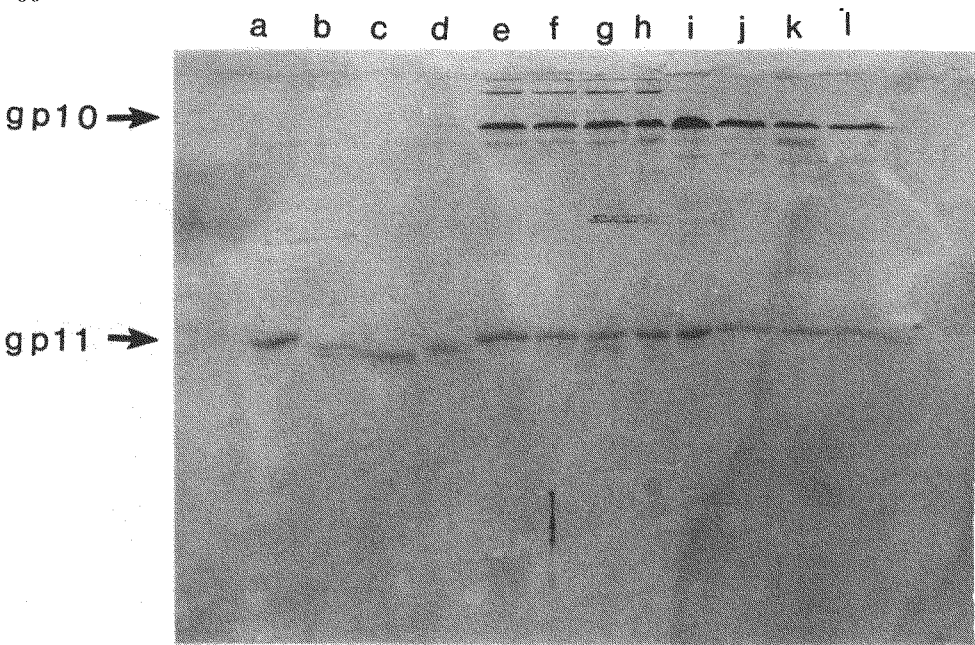


FIG. 2. SDS/polyacrylamide gel electrophoresis analysis of protease effects. Samples of 5  $\mu\text{g}$  of P11 (lanes a to d), 9  $\mu\text{g}$  of *in vitro*-formed P(10/11) complex (lanes e to h) and 9  $\mu\text{g}$  of *in vivo*-formed P(10/11) complex (lanes i to l) were treated with trypsin (lanes b, f and j), subtilisin (lanes c, g and k) or carboxypeptidase Y (lanes d, h and i) as described for Fig. 2, for 90 min. The samples were examined on a 0.1% SDS/12.5% polyacrylamide gel containing 6 M-urea. Control samples of P11 (lane a), *in vitro*-formed P(10/11) (lane e), and *in vivo*-formed P(10/11) (lane i) were also run on this gel. The mobilities of gp10 and gp11 subunits are indicated on the left.

complex, and the P(10/11) complex itself appears to be resistant to proteolytic action.

Limit trypsin digestion samples of P11 were also examined on a HPLC sizing column. An untreated sample of P11 elutes from the column at a calculated molecular weight of 58,000, in agreement with the reported dimeric molecular weight of 60,000 (Plishker *et al.*, 1983). The trypsin-treated sample elutes at a molecular weight of 53,000. This suggests that trypsin-treated P11 still exists as a dimeric molecule, although each of the gp11 subunits has lost approximately 2000 to 3000 in  $M_r$ . This is in agreement with the results shown in Figure 2.

The apparent similarities in the native electrophoresis patterns created by subtilisin, trypsin and carboxypeptidase Y, as described above, led us to compare these patterns on a single gel. Samples from each time-point in each of the digestion time-courses (see Fig. 1) were combined and examined as single samples by non-denaturing gel electrophoresis (Fig. 3). Lanes a and e contain untreated samples of P11. Lane b contains a combination of the samples from a time-course of incubation of P11 with trypsin, lane c from an incubation with subtilisin, and lane d from an incubation with carboxypeptidase Y. The banding patterns are similar, suggesting that these proteases act in a similar, but not identical, fashion

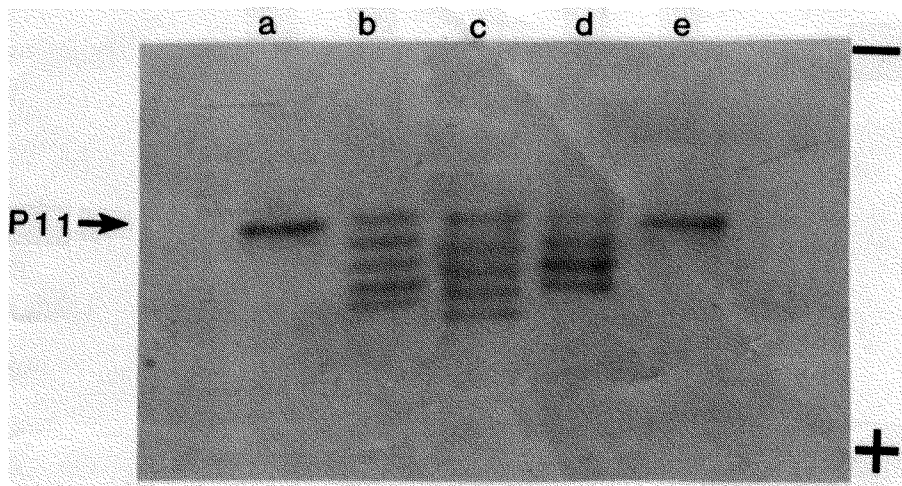


FIG. 3. Effect of protease treatment on P11 mobility. Samples of P11 (10  $\mu$ g) were digested as described for Fig. 1 with trypsin (50 ng) (lane b), subtilisin (50 ng) (lane c) or carboxypeptidase Y (400 ng) (lane d). A 1- $\mu$ g portion of each of the reaction mixtures was removed at 0, 1, 15, 60, 90 and 180 min, and PMSF was added to stop the digestion. These samples were combined and examined as a single sample on a 7.5% non-denaturing polyacrylamide gel. Lanes a and e contain untreated samples of P11.

to change the structure of P11. The results of these experiments suggest that the individual bands arise from charge differences, each more rapidly migrating band resulting from the loss of one net positive charge from the band immediately above it. Removal of the first positive charge in each case is diagnostic of the complete loss of P11 assembly activity, however, this activity may be lost before the charged amino acid has been removed.

If these proteases are acting only at the carboxyl end of the gp11 subunits, then no new amino termini should be generated on the core of P11 left after limit digestion. Limit digestion samples of P11 treated with trypsin, subtilisin or carboxypeptidase Y were chromatographed on Sephadex G-50, and the isolated P11 cores were reacted with dansyl chloride, hydrolyzed and analyzed as described in Materials and Methods. The chromatograms of isolated control P11 and the digested cores were essentially identical. *O*-dansyl-tyrosine and di-dansyl-tyrosine were present as the most intense spots in all four samples (data not shown). Epsilon-dansyl lysine was present in small amounts, with only trace amounts of fluorescence observed for other dansyl derivatives. It appears that the amino-terminal amino acid of gp11 is tyrosine and that no new amino-termini are generated by these three proteases under the conditions described above.

### (c) Analysis of the carboxy terminus of gp11

Since it appears that the hydrolysis by these proteases is limited to the removal of only 20 to 30 amino acids from the carboxyl-terminal portion of the gp11 subunit, we attempted to determine the nature of this removed portion. A sample

of P11, which had been treated with carboxypeptidase Y for 90 minutes, was subjected to amino acid analysis to determine the amino acids that had been released. This sample was also analyzed by native polyacrylamide gel electrophoresis and was shown to be void of the native P11 band and to contain all three of the more rapidly migrating bands shown in Figure 3. The tryptic peptides in a limit digest sample of P11 were isolated as described in Materials and Methods. This peptide mixture was hydrolyzed and subjected to amino acid analysis. The data from the analysis of carboxypeptidase Y-treated and trypsin-treated samples (not shown) indicate that the amino acids released by carboxypeptidase Y can be accounted for in the composition of the tryptic peptide mixture. The portion of gp11 removed by the proteases may indeed come from the same region of the gp11 molecule.

A time-course analysis of the amino acids released by carboxypeptidase Y was performed on P11 as described in Materials and Methods (data not shown). The first amino acids released suggest that the sequence of amino acids at the carboxy terminus of gp11 may be -Ala-Leu-Val-COOH. At least 10 additional aliphatic and aromatic amino acids are released before the first basic amino acid (Lys) is removed by carboxypeptidase Y.

#### 4. Discussion

The assembly of the 1/6th arm of the baseplate of bacteriophage T4 is an example of a linear ordered assembly pathway regulated at the level of protein-protein interactions rather than at the level of gene expression. The regulation involves interactions between the growing structure and the soluble protein subunits rather than between the subunits themselves. During the assembly process the subunits must be switched from non-reactive precursor conformations to reactive conformations. An example of changes in subunit conformation and reactivity is suggested in the assembly of catalytic trimers of aspartate transcarbamoylase (Burns & Schachman, 1982*a,b*). A description of these subunit protein-protein interactions on a molecular level would include defining those domains of subunit proteins or specific amino acids that are involved in assembly interactions. Regions (or amino acid residues) of the protein that become more (or less) accessible to proteases, chemical reagents, etc., during the assembly reactions would be postulated to be involved in the switching event or in the assembly site itself. Newly exposed regions would be expected to be involved in the formation of assembly sites where the next protein would add to the growing structure. Loss of assembly activity by modification or proteolytic removal of these regions would be further proof of their importance in the assembly function of these proteins. We have used this last approach, proteolytic removal of a portion of an assembly protein, to determine the region of P11 essential for assembly activity.

The first step in a direct approach to understanding the protein-protein interactions in the assembly of the T4 baseplate wedge structure was the isolation and characterization of the first assembly intermediate, the P(10/11) complex (Berget & King, 1978). The formation of the first intermediate in this 1/6th arm assembly pathway involves the interaction of the phage proteins P10 and P11.

These two proteins were separately isolated from cells infected with mutant strains of T4 defective in the production of the other protein such that assembly does not occur *in vivo*. Thus, they are assembly "naive" (Plishker *et al.*, 1983). The isolated proteins interact *in vitro* to form a species that, when analyzed by non-denaturing gel electrophoresis is identical to the P(10/11) complex formed *in vivo* (Plishker *et al.*, 1983). The appearance of this P(10/11) band in the non-denaturing gel system has been used as an assay for the assembly activity of P10 and P11.

Limited proteolysis has been used in a number of cases to separate proteins into domains, each possessing some function of the undigested molecule. Proteolysis of CAP protein ( ; Krakow & Pastan, 1973) and of phage lambda repressor (Pabo *et al.*, 1979) was used to define the DNA-binding domains of these two DNA-binding proteins, the carboxyl-terminal portion of CAP and the amino-terminal part of repressor being the regions involved. Proteolytic treatment of both aldolase and fructose 1,6-bisphosphate suggested that the interaction between these two proteins involves the carboxyl-terminal region of the former and an internal region of the latter (Pontremoli *et al.*, 1982). The hemoglobin-binding site on haptoglobin heavy chain was also delineated by selective proteolysis (Lustbader *et al.*, 1983). We have treated both P10 and P11 with proteases in an attempt to determine domains in either protein that are involved in their assembly. Treatment of P10 with a variety of proteases had no detectable effect on the ability of this protein to interact with P11. However, treatment of P11 under native conditions with subtilisin, trypsin or carboxypeptidase Y resulted in a time-dependent loss in the assembly activity of P11. These treatments did not result in complete hydrolysis of P11 into peptides; instead, there was a loss of approximately 2000 to 3000 g/mol from the gp11 subunit. There was no apparent change in P11 when the P(10/11) complex was treated with these proteases. Dansylation of untreated P11 and of the P11 "cores" remaining after treatment with each protease resulted in the formation of only dansyl-tyrosine derivatives. These data suggest that a portion of the carboxyl end of the gp11 subunit is uniquely removed by these proteases, and that this portion of the molecule is involved in the interaction of P11 with P10.

Concomitant with loss of assembly activity, there was a change in the mobility of P11 on non-denaturing polyacrylamide gels from a single band to a series of more mobile bands. The banding patterns created by protease treatment suggest that the bands represent the removal of single positive charges, each band representing the loss of one charge from the band directly above it. Although the disappearance of the native band in native gels is diagnostic of the loss of P11 assembly activity, assembly activity may in fact be lost before the native band disappears (Fig. 1, lanes g to i). In these *in vitro* assembly reactions performed with subtilisin-treated P11 and excess P10, more residual "native" P11 remains unassembled (lanes h and i) compared to untreated P11 (lane g). Thus the removal of all or some of the 13 uncharged carboxyl-terminal amino acids before the first positive charge is removed may be sufficient to inactivate P11. The native polyacrylamide gel system used in this study is surprisingly sensitive in the detection of the proteolysis of P11. Although we propose that the changes in

mobility of P11 are primarily due to net positive charge removal, the change in mass also affects the migration of P11 in this system. The similarity of the mobility of the trypsin and carboxypeptidase Y-generated bands (Fig. 3, lanes b and d) may be explained by the specificities of these two proteases. Carboxypeptidase Y removes carboxyl-terminal basic amino acids (Lys and Arg) at a much reduced rate compared to other amino acids (Hayashi *et al.*, 1975) and thus any transient P11 intermediates in this proteolysis reaction should bear a basic amino acid at their carboxyl termini. Trypsin also leaves a basic amino acid at the carboxyl termini of peptides that it generates. Subtilisin (lane c) generates a similar ladder pattern; however, the exact mobility of each of the bands is different from that for trypsin or carboxypeptidase Y. This is no doubt caused by the different specificity of subtilisin and most probably results from the removal of additional amino acid residues amino-terminal to the basic amino acids left by trypsin or carboxypeptidase Y. Trypsin and subtilisin appear to remove two positive charges from each of the carboxyl termini of the two gp11 subunits in the P11 molecule during the course of the incubation. Carboxypeptidase Y seems able to remove only three of these four positive charges.

The assembly of P10 and P11 in the formation of the baseplate is not one of the uniquely ordered assembly reactions discussed in the introduction (Edgar & Lielausis, 1968). The product of gene 11 can assemble into a structure at any stage in the morphogenesis of the baseplate or of the entire virus particle. Nevertheless, in mutant infections that are blocked at the next gene-controlled step (gene 7), all of the P11 is in a complex with P10 (Berget & King, 1978). The immediate interaction of P11 with P10 during the normal assembly of baseplate precursors would lessen the chances of *in vivo* proteolysis of the carboxyl termini of P11. Clearly, subunits that have been damaged by proteolysis would be unwanted participants in the assembly of multi-protein aggregates, for their function may be impaired. In such assembly proteins, the active sites of assembly may be the sites most susceptible to proteases, thus guaranteeing that any protease-generated damage would first remove the subunit from the assembly scheme.

An understanding of the control mechanisms of the ordered assembly reactions in T4 baseplate morphogenesis will come from a close study of the succeeding steps in this assembly pathway. The techniques we have developed in the study of P(10/11) complex formation should be applicable to the study of these assembly reactions.

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