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Precursors of the T4 Internal Peptides

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The precursors of the two T4 internal peptides have been identified by in vitro cleavage of individual phage proteins eluted from sodium dodecyl sulfate-acrylamide gels. The precursor of internal peptide VII is p22, the product of T4 gene 22 and an essential component of the morphogenic core. The precursor of peptide II is a protein with a molecular weight of approximately 13,000, whose gene has yet to be defined by mutation. A newly detected protein of approximately 15,000 molecular weight is found to be cleaved and is, therefore, likely to be a component of precursor head structures.

Assembly of the head of bacteriophage T4 is thought to be initiated by a morphogenic core composed of several phage-coded proteins (17, 19, 20). The known components of this core are the protein product of gene 22 (p22) and three nonessential proteins (IP-I, IP-II, and IP-III). After the capsid proteins are assembled around the core, each of the four core proteins undergoes proteolytic cleavage (12, 15, 24). The three nonessential proteins are thereby transformed, by removal of short N-terminal segments, becoming the internal proteins of the mature phage particle (15, 24), whereas p22 is extensively degraded (9, 15).

None of the small peptide fragments of these cleavage reactions have previously been detected by acrylamide gel electrophoretic techniques. Two cleavage fragments contained inside the head of the mature phage particle (8) have, however, been detected and characterized by other methods (2, 3). These internal peptides, designated II and VII, have respective molecular weights of 3,900 and 2,500 and are unusually rich in acidic residues (3). It has been suggested that these peptides may play an active role in the packaging of DNA in the phage head (16, 17).

That the principal core protein p22 is extensively degraded suggests the possibility that the internal peptides are fragments of p22. In a previous report (6), indirect evidence was presented in support of this precursor-product relationship in the case of peptide VII. The same studies indicated, however, that p22 is not the precursor of peptide II. Furthermore, neither of three internal protein precursors can be the precursor of peptide II, as shown by the dissimilarity of the composition of peptide II as compared with the compositions of the internal protein cleavage fragments (2, 12, 24). The known components of the morphogenic core are thus not sufficient to account for peptide II.

The present report describes in vitro cleavage studies with purified phage proteins that directly confirm p22 as the precursor of peptide VII and identify the precursor of peptide II.

MATERIALS AND METHODS

Phage, bacteria, and nomenclature. The following phage mutants used in this study were derived from T4D and have been described previously (4, 5, 13, 14, 21): *am*E1137 in gene 19, *am*N90 in gene 21, *am*E322 in gene 21, *ts*N8 in gene 21, *am*B270 in gene 22, *am*E198 in gene 57, and *am*H26*am*H11 in genes *e* and 23. The mutant *am*111 in gene 21 was derived from T2L (R. L. Russell, Ph.D. thesis, California Institute of Technology, Pasadena, 1967). When useful, gene designations will be given in parentheses after the mutant number, e.g., *am*E322(21$^-$).

The permissive hosts used for amber mutants were *Escherichia coli* CR63 for T4D-derived mutants and 011′ for T2L-derived mutants. *E. coli* BB was used as the host for temperature-sensitive mutants (permissive temperature 30°C, restrictive temperature 42°C) and as the restrictive host for all amber mutants.

Phage mutants blocked in the early steps of head assembly (of genes 20, 21, 22, 23, 24, 31, and 40) are also blocked in assembly-dependent cleavage in vivo and are termed "cleavage negative." Wild-type phage and mutants defective in other late genes are termed "cleavage positive."

^{14}C-labeled lysates for gel electrophoresis. Ten-milliliter cultures of *E. coli* BB were grown to a density of 5 × 10^8 cells/ml in M9 medium (1) at 37°C (for amber mutants) or 42°C (for temperature-sensitive mutants) and infected with a multiplicity of five phage per bacterium. Eight minutes after infection, the cultures were superinfected at the same multiplicity, and, at 10 min, 10 μCi of [^{14}C]lysine (350 Ci/mol) was added. The cells were harvested by centrifugation at 30 min after infection and suspended in 0.4 ml of sample gel buffer (15). The samples were boiled for 2.5 min and stored frozen until use.

Acrylamide gel electrophoresis. Short slab gels (10 cm) containing 10% acrylamide and 0.1% sodium dodecyl sulfate (SDS) were used to isolate the product of gene 22 by the discontinuous buffer system of Laemmli (15). Electrophoresis was performed at 30 mA/gel for 3 h, or until the bromophenol blue tracking dye reached the bottom of the gel.

Long linear gradient gels (28 cm) of 8 to 15% acrylamide containing 0.1% SDS were used to identify and isolate the precursor of peptide II. A 3% stacking gel at pH 6.8 was overlaid on the gradient gel according to the buffer system of Laemmli (15), except that the final concentration of ammonium persulfate was reduced to 0.1 mg/ml to facilitate pouring the gradient. Electrophoresis was performed at 40 mA for 4 to 5 h, using tap water for cooling. The preparative gels of 8 to 15% were run until the bromophenol blue reached the bottom of the gel.

The electrophoretic apparatus (models SE500 and SE520) were manufactured by Hoefer Scientific Instruments, San Francisco, Calif., and were used with 1.5-mm spacers between the gel-forming plates. Generally about 10^5 cpm of ^{14}C-labeled protein in 0.01 ml was added per well for analytical gels and 5×10^5 cpm in 0.035 ml for preparative gels.

Autoradiography. Immediately after electrophoresis, the gels were placed on a backing strip of filter paper and dried with heat under vacuum. The dried gel was placed in contact with Kodak blue sensitive X-ray film for 2 to 4 days.

Elution of proteins from SDS gels. Protein bands were precisely located on the gels by means of orienting radioactive ink markings, excised and eluted by the following modification of the method of Weber et al. (25). The dried pieces of gel on filter paper were rehydrated in 10 to 20 ml of 0.05 M NH_4HCO_3 containing 0.05% SDS and shaken at 37°C overnight. After addition of 1 mg of bovine serum albumin, the gel pieces were incubated an additional 30 min. The resulting solution of eluted protein was lyophilized, after removal of the gel pieces by centrifugation. To remove the bound SDS, the lyophilized material was redissolved in 0.3 M NH_4HCO_3 and precipitated with 90% acetone. The precipitate was washed once with 90% acetone and suspended in 0.1 M Tris-hydrochloride, pH 7.8. This procedure usually solubilizes 80 to 90% of the label from the gels.

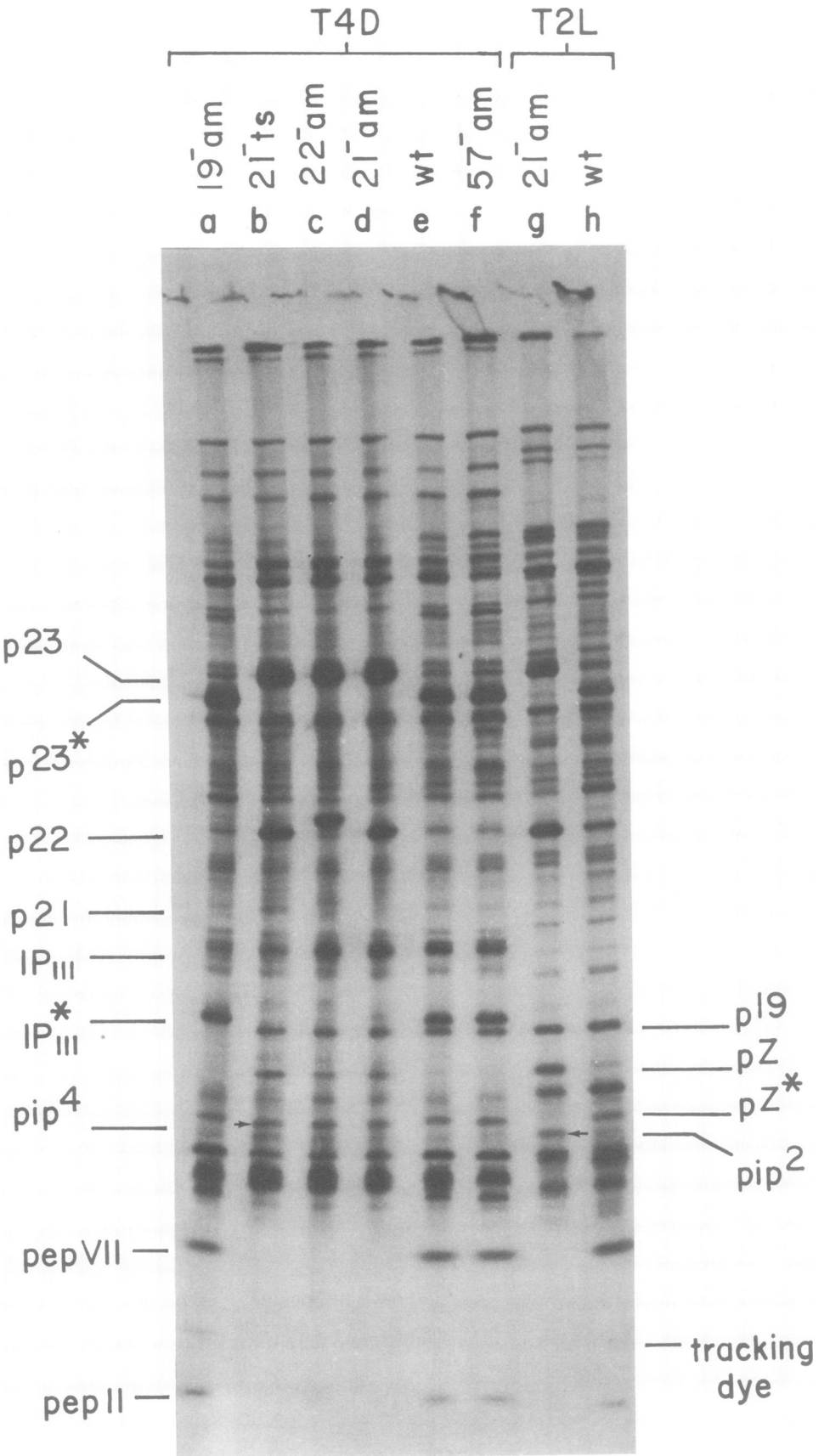
Cleavage of internal peptide precursors. Samples of ^{14}C-labeled proteins eluted from acrylamide gels were subjected to proteolytic cleavage with an extract of *e*$^-$*23*$^-$-infected cells in 0.1 M Tris-hydrochloride, pH 7.8, as the source of cleaving activity. The extract was prepared as described previously (6, 7) and was concentrated to between 30 and 40 mg of protein per ml, as estimated by the Folin-Ciocalteau reaction with bovine serum albumin as a standard. The final concentration of extract protein in the incubation mixture was 20 mg/ml. The substrate-extract mixture was incubated for 6 h at 37°C, at which time dichloroacetic acid (DCA) was added to 10% (7). The ^{14}C-labeled products of the incubation soluble in DCA were analyzed together with ^3H-labeled marker peptides II and VII by gel filtration on Sephadex G50 followed by ion-exchange chromatography on Dowex 50-X2, as described previously (7). Samples were assayed for ^{14}C or ^3H by drying in 5-ml vials. The material was dissolved again in 0.1 ml of water, and, after the addition of 2.0 ml of Hydromix (Yorktown Research), the samples were counted in an Intertechnique SL-30 liquid scintillation counter.

RESULTS

The precursor of internal peptide VII is p22. As described previously (6), the in vitro formation of both internal peptides II and VII can be effected when the total unfractionated protein from cells infected with a phage mutant blocked in cleavage is the substrate. Fractionated substrate protein, highly enriched for p22, was found, however, to yield peptide VII but not peptide II. It was thus concluded that p22 could be the precursor of peptide VII but not of peptide II.

To confirm this finding more directly, we have prepared pure p22 by eluting the appropriate band from an SDS-polyacrylamide gel. An autoradiogram of a typical gel is shown in Fig. 1. After removal of SDS, the denatured ^{14}C-labeled protein was incubated with a cell extract containing the gene 21-dependent cleaving factor (6, 18). The acid-soluble material formed during the incubation was analyzed, together with ^3H-labeled marker peptides II and VII, by sequential chromatography on Sephadex G50 and Dowex 50, as shown in Fig. 2a and b. The chromatograms show that the acid-soluble material includes a component which co-chromatographs with VII on Dowex 50. No in vitro-formed component is seen, however, corresponding to peptide II. Figure 2c shows further that digestion of the presumptive peptide VII with trypsin yields ^{14}C-labeled fragments chromatographically identical to the ^3H-labeled fragments derived from the marker peptide VII.

As reported previously (6), crude, unfractionated substrate protein prepared from cells infected with the mutant *am*E209(22$^-$) fails to yield peptide VII in vitro, whereas VII is formed from substrate prepared from cells infected with the more C-proximal mutant *am*B270(22$^-$). The gel pattern of Fig. 1c shows the fragment of p22 produced by *am*B270. This fragment is displaced slightly upward relative to wild-type p22, thus revealing a minor protein that comigrates with wild-type p22. To eliminate the possibility that this minor protein, rather than p22, is the true precursor of peptide VII and to confirm the results with unfractionated substrate, the *am*B270 fragment of p22 was eluted from the gel and digested, as in the


T4D
T2L
a 19⁻am
b 21⁻ts
c 22⁻am
d 21⁻am
e wt
f 57⁻am
g 21⁻am
h wt
p23
p23*
p22
p21
IP III
IP III*
pip4
pepVII
pepII
p19
pZ
pZ*
pip2
tracking dye

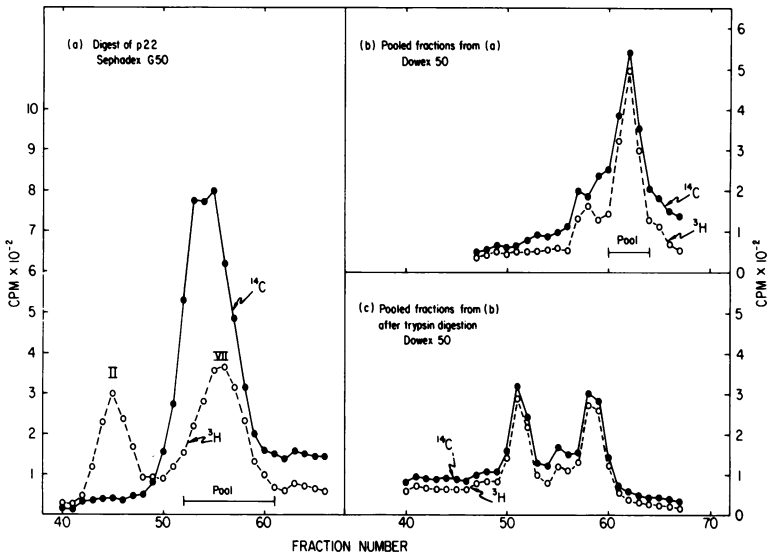


FIG. 2. *Cleavage of peptide VII from p22. [^{14}C]lysine-labeled p22 was obtained by electrophoresis of the proteins from amN90-infected cells on an SDS-acrylamide (10%) slab gel followed by elution of the appropriate band as described in the text. The SDS-freed precipitate was suspended in 0.1 M Tris-hydrochloride, pH 7.8, and incubated with an extract of e$^-$23$^-$-infected cells for 6 h at 37°C. The products of the incubation soluble in 10% DCA were analyzed as shown: (a) analysis of acid-soluble incubation products on Sephadex G50, (b) rechromatography on Dowex 50 of material from (a) eluting with ^{3}H-marker peptide VII, (c) rechromatography on Dowex 50 of pooled material from (b) after digestion with trypsin (50 μg in 0.1 M Tris-hydrochloride, pH 8.0, per ml at 37°C for 9 h).*

experiment of Fig. 2. Again peptide VII was detected among the digestion products. These experiments show conclusively that p22 is the precursor of internal peptide VII.

As noted previously by Onorato and Showe (18) and Laemmli (15), and as seen in Fig. 1, the position of the *am*B270 fragment of p22 on the gel indicates an apparent molecular weight larger than that of the complete protein. This aberrant migration is not noted, however, on gels containing urea rather than SDS (9).

The electrophoretic conditions used for the acrylamide gel of Fig. 1 were chosen to enhance the resolution of components with molecular weights less than about 30,000. It is to be noted that even internal peptides II (molecular weight, 3,900) and VII (molecular weight, 2,500) are visible and resolved well on this gel, although their order is reversed from that expected based on molecular weight. Peptide II, in fact, migrates well ahead of the tracking dye and, for this reason, eluded detection in earlier studies. The aberrant behavior of peptide II on the gel is most likely due to its highly acidic character (2).

Precursor of peptide II. Since little, if any, protein fails to enter the gel shown in Fig. 1, at least one of the bands on such a gel must correspond to the precursor of peptide II. To identify which of the components is the peptide II precursor, an SDS gel similar to that of Fig. 1c was cut into four segments corresponding to the upper, middle, and lower thirds of the gel, and the band at the gel front, respectively. The protein from each segment was eluted, freed of SDS, and used as substrate in the in vitro cleavage assay for peptide II. As shown in Fig. 3, only the lower third of the gel contains material from which peptide II can be derived. The bands in this portion of the gel (molecular weight of approximately 10,000 to 18,000) were then tested individually, resulting finally in the identification of the peptide II precursor as the component labeled pip^4 (*p*recursor of *i*nternal *p*eptide) in Fig. 1. This band has a molecular weight of 13,000, as judged by its almost

FIG. 1. *Autoradiogram of SDS-acrylamide (8-15%) gradient gel of [^{14}C]lysine-labeled proteins from cells infected with various T4D (a through f) and T2L (g through h) mutants: a, amE1137(19$^-$); b, tsN8(21$^-$); c, amB270(22$^-$); d, amE322(21$^-$); e, T4D wild type; f, amE198(57$^-$); g, am111(21$^-$); and h, T2L wild type. Molecular weight estimates (in parentheses) from 10% gels (15): p23(56,000), p23*(46,500), p22(31,000), IP-III(23,500), IP-III*(21,000), and p19(18,000).*

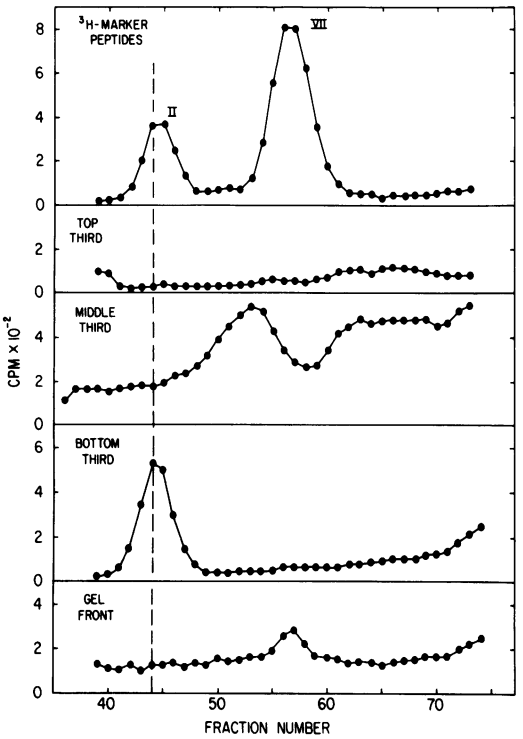


FIG. 3. *Localization of the precursor of internal peptide II on an SDS-acrylamide gel. The [^{14}C]lysine-labeled proteins from amB270-infected cells were fractionated on an SDS-acrylamide (10%) gel. After drying and autoradiography, the gel was cut into the indicated segments from which the proteins were eluted and digested with an extract of e$^-$23$^-$-infected cells as described in the text. The products of the incubation soluble in 10% DCA were analyzed by chromatography on Sephadex G-50 as shown. The elution profiles of the ^{3}H-labeled marker peptides, present in each sample, were essentially identical to the one shown. The precursor of peptide II, present in the lower third of this gel, was identified with a particular band using a higher-resolution, 8 to 15% gradient gel as in Fig. 1.*

exact comigration with equine heart cytochrome *c* (molecular weight, 12,400). Since the pip protein is likely to be highly acidic, this molecular weight should be considered only as a nominal value.

The pip^4 protein is expected to be absent from cleavage-positive extracts. That this is the case is not obvious from Fig. 1 because of the presence, in T4 cleavage-positive extracts (Fig. 1a, e, and f), of a protein designated pZ* that migrates almost in coincidence with pip^4. This protein is distinct from pip^4 since, when cut from the gel of a cleavage-positive extract, it does not yield peptide II upon digestion.

Figure 1 includes gel patterns of T2 proteins derived from cleavage-negative (Fig. 1g) and cleavage-positive (Fig. 1h) infections. The peptide II precursor of T2 (pip^2) is seen to migrate slightly ahead of pip^4, thus better resolving it from pZ* and showing more clearly the absence of pip^2 from the cleavage-positive extract.

That T2 and T4 produce chromatographically distinct species of peptide II (23) can be used to confirm that the product of the in vitro reaction is, in fact, peptide II (7). Thus, as shown in Fig. 4 and 5, the peptide derived from pip^4 elutes on Dowex 50 in coincidence with marker peptide II^4, and the peptide derived from pip^2 elutes in coincidence with the marker peptide II^2.

A newly detected cleaved protein. Since the protein pZ* is present only in cleavage-positive extracts, it is probably derived by cleavage from a larger protein. The most obvious candidate for the precursor of pZ* visible in Fig. 1 is the protein designated pZ, which is present only in cleavage-negative extracts (Fig. 1b, c, d, and g). Although this must be shown directly, it is clear that pZ is a protein whose cleavage is dependent upon head assembly. This protein, which is made by both T2 and T4, is thus most likely to be a component of precursor head structures, as are all other known T4-cleaved proteins.

In vitro yields of the internal peptides. In mature phage particles, the two internal peptides are present in approximately equimolar amounts (2). Table 1 shows the yield of each peptide obtained in vitro by digestion of its respective precursor after eluting the bands from an SDS gel similar to that of Fig. 1. It is seen that the yield of peptide II is 44% the yield

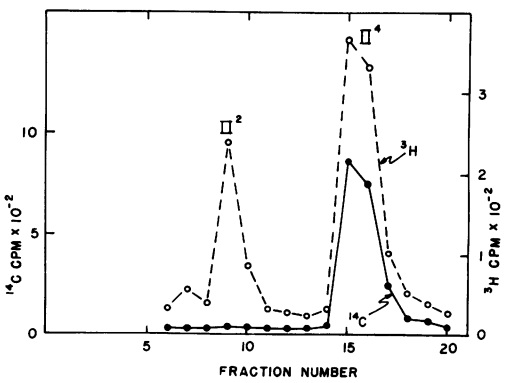


FIG. 4. *Chromatography on Dowex 50 of the ^{14}C-labeled peptide derived from pip^4. The band designated pip^4 in Fig. 1 was eluted from a separate but identical gel and incubated with an extract of e$^-$23$^-$-infected cells. The ^{14}C products of the incubation soluble in 10% DCA were mixed with ^{3}H-labeled marker peptides II^2 and II^4 and analyzed by chromatography on Dowex 50 as shown.*

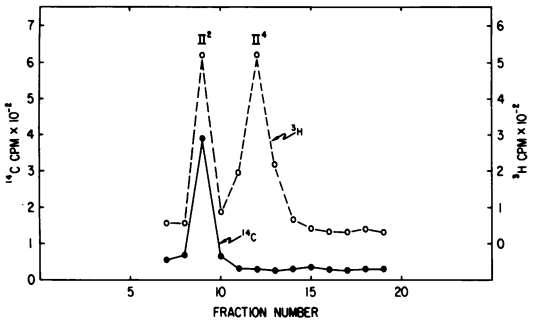


FIG. 5. *Chromatography on Dowex 50 of the ^{14}C-labeled peptide derived from pip². The procedure is identical to that described in the legend of Fig. 4.*

of peptide VII, in terms of [^{14}C]lysine label. Since the lysine content of peptide VII is twice that of peptide II, the in vitro yields are near to equimolar. Although an accurate comparison has not been made, the absolute in vitro yields of the two peptides are also comparable (within a factor of two) to the amounts formed in vivo by T4 wild-type infected cells.

That the two internal peptides are yielded in vitro in approximately equimolar amounts, as they are in vivo, does not necessarily imply that their respective precursors are present in equimolar amounts. It is to be noted from Table 1 that the fraction of label in p22 recovered as peptide VII is only 7%, whereas the maximum value expected is 24%, based on the known lysine content of peptide VII (six residues) and of p22 (25 residues) (24). The equimolar yield of the two peptides in vitro thus depends on the fact that the yield of VII is less than its theoretical maximum. The low yield of peptide VII from p22 in vitro is not due simply to the failure of a large fraction of the p22 molecules to be digested, since better than 75% of the label in p22 is rendered acid soluble. Whether or not a given molecule of p22 yields peptide VII may depend upon the temporal sequence in which the peptide bonds of the molecule are cleaved.

Genetic determinant of the peptide II precursor. Sternberg and Champe (23) used the chromatographic difference of peptide II between T2 and T4 in marker-rescue experiments to map the genetic determinant for this species difference. The determinant was found to be located in the immediate neighborhood of gene 21. At that time, it was argued that the genetic determinant so mapped could correspond either to the structural gene for the precursor of peptide II or, alternatively, to the gene specifying the enzyme that cleaves peptide II from its precursor. Subsequent studies have shown, however, that the specific form of peptide II

TABLE 1. *In vitro yield of the internal peptides*

Protein or peptide	Yield (cpm)	Yield (%)
Input to gel	2.0×10^6	
Eluted p22	8.1×10^4	
Eluted pip	2.2×10^4	
Yield of VII from p22	5.5×10^3	
Yield of II from pip	2.4×10^3	
VII/p22		7
II/pip		11
II/VII		44

obtained by in vitro cleavage depends on the source of substrate protein rather than the source of the cleaving factor (7).

Not only does peptide II show a species difference, but, as pointed out above, its respective T2 and T4 precursors differ as well, as evidenced by the apparent lower molecular weight of pip² as compared with pip⁴ in Fig. 1. This electrophoretic difference of the precursors can likewise be used as a genetic marker in rescue experiments. Accordingly, we have screened am^+ recombinants from T4 $21^-am \times T2am^+$ crosses and found that all (six out of six) that produce the T2-specific peptide II also produce the T2-specific precursor of peptide II. Thus, the genetic determinant for the species difference located close to gene 21 almost certainly represents the structural gene for the peptide II precursor rather than a gene involved in some kind of post-translational modification of the precursor.

A direct test of the possibility that p21 is the precursor of peptide II had not heretofore been possible, because no band had been identified by gel electrophoresis as p21. Recently, however, Showe et al. (21, 22) and Castillo et al. (1a) have identified a 27,000-molecular-weight protein as the immediate product of gene 21. According to Showe et al. (22), this protein accumulates for 21^-ts mutants, but it cleaves to several lower-molecular-weight forms for wild-type T4 and for most other phage mutants blocked in head assembly. This protein could thus conceivably be a higher-molecular-weight precursor of the 13,000-molecular-weight peptide II precursor identified in Fig. 1.

We have also detected a component with a molecular weight of approximately 27,000 that accumulates in cells infected with the 21^- mutant *ts*N8, as shown in Fig. 1b. This protein was eluted from the gel and tested for its ability to yield peptide II. No detectable peptide II was obtained. A sample of the 27,500-molecular-weight protein, provided by Michael Showe, was also tested similarly with the same result.

Furthermore, we have been unable to detect any alteration in the electrophoretic mobility of the pip protein resulting from 21⁻ amber mutations, including the most N-proximal mutation *am*E322 (Fig. 1d). These experiments lead us to conclude that the precursor of peptide II is not the product of gene 21.

The recently discovered nonessential soc protein of the T4 capsid (11) has a molecular weight slightly lower than that of the peptide II precursor, suggesting that the soc protein and peptide II could be derived from a common precursor. A deletion mutant lacking the soc protein, kindly supplied by M. Yanagida, was found, however, to produce peptide II in vivo.

At the present time, the pip protein cannot be identified with a known T4 gene product.

DISCUSSION

The present results show that the two internal peptides of T4 are derived from separate precursors. The precursor of internal peptide VII is the protein product of gene 22. The precursor of internal peptide II is neither p22 nor p21, but a 13,000-molecular-weight protein (pip) whose gene is yet to be defined by mutation. Although the *pip* gene and gene 21 appear to be closely linked, the nonidentity of the two genes is indicated by the absence of an amber mutation in gene 21 that affects the electrophoretic mobility of the pip protein. Furthermore, the protein identified as p21 (22) does not yield peptide II in vitro.

Using a new immunoreplicate technique, Showe et al. (21, 22) have identified the gene 21-dependent proteinase responsible for the cleavage of most, if not all, of the T4 proteins known to be cleaved. The active factor, termed T4PPase, is an 18,000-molecular-weight cleavage fragment of the 27,500-molecular-weight p21 and is found in association with precursor head structures. With [^{14}C]lysine-labeled extracts, we are unable to detect a component corresponding to T4PPase. On the gel of Fig. 1, this protein should be present in the *am*B270(22⁻) extract (Fig. 1c) but absent from the *am*E322(21⁻) extract (Fig. 1d). If T4PPase has a low lysine content, its presence would probably not be detected on our gels. That this may be the case is indicated since the presumptive precursor (p21) of T4PPase is itself only weakly labeled by [^{14}C]lysine. Also, since T4PPase is a catalytic gene product, it may be present in lower amounts than other proteins.

Our previous finding (6) that in vitro formation of both internal peptides requires a functional gene 21 product is consistent with the p21 origin of T4PPase. One point of difference between our results and those of Showe et al. (21) is their finding that purified T4PPase will degrade native but not heat- or acid-denatured p22. Our experiments, by contrast, demonstrate efficient cleavage of p22 extracted from an SDS gel. Moreover, we have previously shown (6) that both internal peptides can be formed in vitro from acid-denatured substrate protein. Since the only discernible difference between the conditions is the use of purified T4PPase versus a crude cell extract, this discrepancy suggests that other factors present in our crude extracts sensitize denatured p22 to the action of T4PPase.

Since peptide II, like peptide VII, is an internal component of mature phage particles, precursor head structures would be expected to contain the pip protein. Experiments are in progress to confirm that this is the case and to determine whether the pip protein is a structural component of the capsid or is associated with the morphogenic core.

An understanding of the role of the pip protein in T4 assembly is hindered by the lack of mutants affected in the *pip* gene. Two features of the pip protein are, however, noteworthy. First, Onorato and Showe (18) have found that both internal peptides II and VII strongly interfere with the precipitin reaction between p22 and anti-p22. As they point out, this interference could reflect binding of the peptide to the p22 antigen as well as to the antibody. In light of our finding that p22 is not the precursor of peptide II, the interference of this peptide in the precipitin reaction can best be explained by an interaction of peptide II with p22. This suggests that the pip protein and p22 may also interact. Second, as described elsewhere (10), a highly acidic internal component analogous to peptide II of T4 has been found in all T-even-related phage strains so far examined, including T2 and T6, and eight of the more recently isolated RB strains (R. L. Russell, Ph.D. thesis, California Institute of Technology, Pasadena, 1967). The ubiquity of a peptide II-like component suggests that the pip protein may play an essential role in the assembly of T-even phage.

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LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophages, p. 446. Interscience Publishers, New York.

1a. **Castillo, C. J., C. Hsiao, P. Coon, and L. W. Black.**

1977. Identification and properties of bacteriophage T4 capsid-formation gene products. J. Mol. Biol. 110:585-601.
2. **Champe, S. P., and H. Eddleman.** 1967. Polypeptides associated with morphogenic defects in bacteriophage T4, p. 55-70. *In* J. S. Colter and W. Paranchych (ed.), The molecular biology of viruses. Academic Press Inc., New York.
3. **Eddleman, H., and S. P. Champe.** 1966. Components in T4-infected cells associated with phage assembly. Virology 30:471-481.
4. **Edgar, R. S., G. H. Denhardt, and R. H. Epstein.** 1964. A comparative study of conditional lethal mutations of bacteriophage T4D. Genetics 49:635-648.
5. **Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R. S. Edgar, M. Susman, G. H. Denhardt, and A. Lielausis.** 1963. Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. 28:375-394.
6. **Giri, J. G., J. E. McCullough, and S. P. Champe.** 1976. Identification of gene products required for in vitro formation of the internal peptides of bacteriophage T4. J. Virol. 18:894-903.
7. **Goldstein, J., and S. P. Champe.** 1974. T4-induced activity required for specific cleavage of a bacteriophage protein in vitro. J. Virol. 13:419-427.
8. **Hershey, A. D.** 1957. Some minor components of bacteriophage T2 particles. Virology 4:237-264.
9. **Hosoda, J., and C. Levinthal.** 1968. Protein synthesis in *Escherichia coli* infected with bacteriophage T4D. Virology 34:709-727.
10. **Howard, G. W., M. L. Wolin, and S. P. Champe.** 1972. Diversity of phage internal components among members of the T-even group. Trans. N. Y. Acad. Sci. 34:36-51.
11. **Ishii, T., and M. Yanagida.** 1975. Molecular organization of the shell of the T-even bacteriophage head. J. Mol. Biol. 97:655-660.
12. **Isobe, T., L. W. Black, and A. Tsugita.** 1976. Primary structure of bacteriophage T4 internal protein II and characterization of the cleavage upon phage maturation. J. Mol. Biol. 102:349-365.
13. **King, J.** 1971. Bacteriophage T4 tail assembly: four steps in core formation. J. Mol. Biol. 58:693-709.
14. **King, J., and U. K. Laemmli.** 1971. Polypeptides of the tail fibers of bacteriophage T4. J. Mol. Biol. 62:465-477.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) 227:680-685.
16. **Laemmli, U. K., and M. Favre.** 1973. Maturation of the head of bacteriophage T4. I. DNA packaging events. J. Mol. Biol. 80:575-599.
17. **Laemmli, U. K., J. R. Paulson, and V. Hitchins.** 1974. Maturation of the head of bacteriophage T4. V. A possible DNA packaging mechanism: *in vitro* cleavage of the head proteins and structure of the core of the polyhead. J. Supramol. Struct. 2:276-301.
18. **Onorato, L., and M. K. Showe.** 1975. Gene 21 protein-dependent proteolysis *in vitro* of purified gene 22 product of bacteriophage T4. J. Mol. Biol. 92:395-412.
19. **Paulson, J. R., S. Lazaroff, and U. K. Laemmli.** 1976. Head length determination in bacteriophage T4: the role of the core protein P22. J. Mol. Biol. 103:155-173.
20. **Showe, M. K., and L. W. Black.** 1973. Assembly core of bacteriophage T4: an intermediate in head formation. Nature (London) New Biol. 242:70-75.
21. **Showe, M. K., E. Isobe, and L. Onorato.** 1976. Bacteriophage T4 prehead proteinase. I. Purification and properties of a bacteriophage enzyme which cleaves the capsid precursor proteins. J. Mol. Biol. 107:35-54.
22. **Showe, M. K., E. Isobe, and L. Onorato.** 1976. Bacteriophage T4 prehead proteinase. II. Its cleavage from the product of gene 21 and regulation in phage-infected cells. J. Mol. Biol. 107:55-69.
23. **Sternberg, N., and S. P. Champe.** 1969. Genetic determinant of an internal peptide of bacteriophage T4. J. Mol. Biol. 46:377-392.
24. **Tsugita, A., L. W. Black, and M. K. Showe.** 1975. Protein cleavage during virus assembly: characterization of cleavage in T4 phage. J. Mol. Biol. 98:271-275.
25. **Weber, K., J. R. Pringle, and M. Osborne.** 1972. Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. Methods Enzymol. 36:3-28.