The Head–Tail Linker Protein of Bacteriophage T5: Genetic and Immunological Studies

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SUMMARY

We investigated the properties of amber mutants of coliphage T5 defective in a late stage of phage assembly. The non-functional heads synthesized by mutants am25 or am158 were unable to combine with functional tails to produce viable phage particles. These mutants were shown to lack a single protein, designated the head–tail linker protein (HTLp), which was identified by polyacrylamide gel electrophoresis and Western blot analysis and had an Mr of 18,000. An extract containing the HTLp, when added to the HTLp-deficient heads, restored their ability to combine with functional tails.

INTRODUCTION

The bacteriophage T5 is a coliphage with a particle mol. wt. of 10^9 × 10^6 (Dubin et al., 1970). The icosahedral head contains a single molecule of dsDNA 121.3 kbp in length with an 8% terminal repetition (Rhoades, 1982). T5 possesses a non-contractile tail, 200 nm long, to which three L-shaped tail fibres (LTF) and one straight tail fibre (STF) are attached at the distal end (Saigo, 1978). The major structural proteins of the head and tail have been identified and their relative molecular masses determined by SDS–PAGE. A total of six head proteins and nine tail proteins have been described (Zweig & Cummings, 1973; Heller, 1984).

Complete phage heads filled with DNA and functional tails are assembled by independent pathways (Lunt & Kay, 1968). These functional phage substructures are able to complement each other in vitro to form viable phage particles (Zweig & Cummings, 1973). Two amber mutants, both in the same complementation group, have been identified which produce heads filled with DNA and functional tails, but the heads are unable to combine with functional tails to produce viable phage particles in an in vitro complementation test (Kay & Wakefield, 1986). We have proposed that this is due to the absence of a head–tail linker protein (HTLp), and in this paper we describe the identification of this protein.

METHODS

Strains of bacteria and bacteriophage. Escherichia coli F (Lanni, 1958) was the non-permissive strain and E. coli HR34 (Lanni & Lanni, 1966) was the permissive strain for growth of amber mutants. The T5 amber mutants used in the present work were characterized by Kay & Wakefield (1986): am25 and am158, complementation group 11, producers of functional tails and non-functional heads in E. coli F; am123, complementation group 20, and am 17, complementation group 8, producers of functional heads but no tails; am28, complementation group 13, producer of functional tails but no heads.

Media and buffers. These were as previously described (Kay & Wakefield, 1986). For infections with T5, media were made 1 mM with CaCl_2. All incubations were carried out at 34 °C.

In vitro complementation. This was done as previously described (Kay & Wakefield, 1986).

Large-scale preparation and purification of phage and amber mutant products. Four 500 ml nutrient broth cultures in 51 flasks were aerated by rotation, grown to 2 × 10^8 cells/ml and infected at an m.o.i. of 4 with either wild-type

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T5 (T5am+) or an amber mutant (am17, am25, am123 or am158). After incubation overnight in the case of T5am+ or 100 min in the case of the amber mutants, the cultures (except T5am+) were treated with chloroform (0.5 ml/l) and stored at 4 °C overnight. After sedimentation of cell debris at 3000 r.p.m. for 15 min at 4 °C, the supernatant was treated with NaCl (27 g/l) and polyethylene glycol (PEG) 6000 (100 g/l) and stored at 4 °C overnight. The precipitate was collected by sedimentation at 10000 r.p.m. for 15 min at 4 °C, resuspended in T5 buffer and freed from PEG 6000 by sedimentation at 10000 r.p.m. followed by washing the sediment with T5 buffer and pooling the phage-containing supernatants. The T5am+ or am mutant product was sedimented at 30000 r.p.m. for 60 min at 4 °C, resuspended in 20 ml T5 buffer. CsCl (19.5 g) was then dissolved in this suspension. After centrifugation at 100000 r.p.m. for 18 h at 20 °C the visible band was removed with a hypodermic needle, diluted with T5 buffer and sedimented at 30000 r.p.m. for 60 min at 4 °C. The pellet was resuspended in T5 buffer (8 ml) and fractionated on sucrose density gradients (15% to 30%, w/v) at 14200 r.p.m. for 60 min at 4 °C in a Beckman SW28 rotor. The position of the phage was recorded using an Isco u.v. analyser and the peak fractions were pooled and stored with 10-2 M-sodium azide. The content of phage or heads was standardized by their u.v. absorption at 260 nm.

Preparation of HTL protein-containing extract. Four 500 ml cultures of E. coli F were grown in nutrient broth to 1.5 x 108 cells/ml and infected with T5am+ phage at an m.o.i. of 5. Incubation was stopped after 110 min (before lysis) and the cells were harvested by centrifugation at 3000 r.p.m. for 15 min at 4 °C. The cells were resuspended in 15 ml of T5 buffer with 10-2 M-sodium azide, centrifuged at 30000 r.p.m. for 60 min at 4 °C and the supernatant was subjected to u.v. light 340 mm below a 15 W germicidal lamp for 5 min.

Antisera. Rabbits were injected intramuscularly three times with 0.5 ml of a mixture of either T5am+ or am17p with Freund's adjuvant at 20 day intervals and bled after a further 20 days. Both the phage and the am17p used were peak fractions from sucrose density gradients with titre or phage equivalent concentrations of 7 x 107/ml. Adsorbed antiserum was prepared by mixing anti-am17p antiserum with an equal volume of sucrose gradient-fractionated am25p, incubating for 60 min at 34 °C and removing remaining am25p and antigen-antibody complexes by centrifugation (30000 r.p.m. 60 min). The process was repeated three times.

Sucrose density centrifugation. This was as described previously (Kay & Wakefield, 1986).

SDS-PAGE. This was performed by the method of Laemmli & Favre (1973).

Western blotting. This was performed as described by Burnette (1981). The first antibody was phage-specific (see text) and the second antibody was radiiodinated horse anti-rabbit immunoglobulin.

RESULTS

In vitro complementation analysis of am25 product

The amber mutants, am25 and am158, have been assigned to complementation group 11 by the in vitro complementation test (Kay & Wakefield, 1986). During infection of the non-permissive host, these mutants produced heads filled with DNA, as detected by sucrose density gradient analysis. In vitro complementation analysis of the products of infection under non-permissive conditions revealed that both am25 and am158 synthesized functional tails but that the head-like particles which had been synthesized were unable to yield infectious particles when treated with functional tails (Kay & Wakefield, 1986). This observation was investigated further and the results are shown in Fig. 1. The products of infection of the non-permissive host were prepared, am17 being chosen as the functional head producer and am28 as the functional tail producer. These structures were used for the in vitro complementation experiments and the products of the complementation were analysed on sucrose density gradients. Fig. 1(a) shows the position of the T5am+ particles in the sucrose density gradient and Fig. 1(b) shows the absence of any peak in the am28 product, am28p (the suffix 'p' after the mutant denotes the product of infection under non-permissive conditions). Fig. 1(c) and 1(d) show the peaks obtained with am17p and am25p, respectively, indicating that both functional heads, am17p, and inactive heads, am25p, run to the same position in the gradient which is lower than that of complete T5am+ particles. Incubation of a mixture of am17p and am28p resulted in a reduction of the peak corresponding to the free heads and the appearance of a peak corresponding to that of T5am+ (Fig. 1(e)). Analysis of the samples for plaque-forming ability showed that the peak of infectivity corresponded to the latter peak. In contrast, when am25p was incubated with am28p, no peak corresponding to complete phage appeared (Fig. 1(f)). Therefore, the heads produced by am25, though indistinguishable by sucrose gradient analysis from those produced by am17, are, in contrast, unable to combine with functional tails to produce complete phage particles. When am25p and am17p were mixed, complete phage particles were produced (Fig. 1(g)). This shows
that am25 synthesizes functional tails which, although unable to combine with its own non-functional heads, can combine with the functional heads of am17.

SDS–PAGE analysis of non-functional heads

PAGE was used to detect any differences between the protein composition of the functional and non-functional heads. In these experiments, am123 was used as the functional head-producer. T5am+*, am25p, am123p and am158p particles were analysed by SDS–PAGE (see Fig. 2a). In order to reveal differences between the minor proteins, some of the major bands were heavily overloaded. The protein bands of the wild-type, numbered according to Zweig & Cummings (1973) and Heller (1984) comprise bands 1 and 2, the tail fibre proteins (M, 125000 and 108000); bands 3 and 4, tail proteins (M, 103000 and 75000); band 5, the receptor-binding protein (M, 67000); band 6, the major tail protein (M, 58000); band 7, a head protein (M, 44000); band 8, the major head protein (M, 32000); band 9, a tail protein (M, 22000); bands 10 and 11, head proteins (M, 19000 and 18000); and bands 12 and 13, tail proteins (M, 17000 and 15000).

Neither am25p, am158p nor am123p contained any of the structural proteins of the phage tail (Fig. 2a), but the non-functional heads, am25p and am158p also lacked the band corresponding to the head protein 11 which was present in the functional heads, am123p. By comparison with the mobility of molecular weight markers, this protein appears to have an M, of 18 000. Since it is absent from heads which are unable to link with functional tails, we propose that it constitutes the HTLP of phage T5.
Fig. 2. Analysis of T5 wild-type and mutant products by SDS–PAGE and immunoblotting. Samples of lane 1, T5-am⁰; lane 2, am25p; lane 3, am123p and lane 4 am158p, purified on sucrose density gradients (see legend to Fig. 1) were analysed on 10% discontinuous SDS–polyacrylamide gels. (a) Coomassie Brilliant Blue-stained gel, (b) Coomassie Brilliant Blue-stained gel at 1/10 loading of (a), (c) Immunoblot of (b) using am25p-adsorbed rabbit anti-am123p serum as first antibody and radioiodinated horse anti-rabbit second antibody.

Table 1. Effect of adsorbed and non-adsorbed serum on wild-type T5 and in vitro complementation between amber mutants

<table>
<thead>
<tr>
<th>Phage or mutant product</th>
<th>Pre-incubation</th>
<th>Second mutant product</th>
<th>Titre (p.f.u./ml)</th>
</tr>
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<tbody>
<tr>
<td>T5am⁰</td>
<td>-</td>
<td>-</td>
<td>3.2 × 10¹⁰</td>
</tr>
<tr>
<td>T5am⁰</td>
<td>Whole serum</td>
<td>-</td>
<td>6.4 × 10⁷</td>
</tr>
<tr>
<td>T5am⁰</td>
<td>Adsorbed serum</td>
<td>-</td>
<td>3.1 × 10⁹</td>
</tr>
<tr>
<td>am123p</td>
<td>-</td>
<td>-</td>
<td>5.9 × 10⁷</td>
</tr>
<tr>
<td>am28p</td>
<td>-</td>
<td>-</td>
<td>2.9 × 10⁸</td>
</tr>
<tr>
<td>am123p</td>
<td>-</td>
<td>am28p</td>
<td>1.6 × 10¹⁰</td>
</tr>
<tr>
<td>am123p</td>
<td>Whole serum</td>
<td>am28p</td>
<td>5.6 × 10⁸</td>
</tr>
<tr>
<td>am123p</td>
<td>Adsorbed serum</td>
<td>am28p</td>
<td>5.0 × 10⁹</td>
</tr>
</tbody>
</table>

The effect of anti-HTLp antibody on in vitro complementation

A serum was raised against purified T5 heads (see Methods). In an attempt to prepare a monospecific antibody against the HTLp, the whole antiserum was adsorbed three times with the HTLp-deficient heads, am25p. The whole serum and the adsorbed serum were tested for the ability to neutralize the infectivity of wild-type T5 (Table 1). Whereas the complete antiserum reduced the titre of T5am⁰ by 98%, the adsorbed serum had no significant effect. Pre-incubation with adsorbed serum of the functional heads, am123p, reduced the efficiency with which they formed viable phage when incubated with excess functional tails, although unadsorbed serum was still more potent than adsorbed serum (Table 1).
Table 2. Conversion of am25p heads to functional form

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Components</th>
<th>Phage titres (p.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>am25p alone</td>
<td>$2.8 \times 10^5$</td>
</tr>
<tr>
<td>2</td>
<td>am28p alone</td>
<td>$2.0 \times 10^3$</td>
</tr>
<tr>
<td>3</td>
<td>Extract alone</td>
<td>$1.2 \times 10^7$</td>
</tr>
<tr>
<td>4</td>
<td>am123p alone</td>
<td>$7.2 \times 10^7$</td>
</tr>
<tr>
<td>5</td>
<td>am28p + am25p</td>
<td>$2.0 \times 10^5$</td>
</tr>
<tr>
<td>6</td>
<td>am28p + am123p</td>
<td>$5.8 \times 10^{10}$</td>
</tr>
<tr>
<td>7</td>
<td>Extract + 28p</td>
<td>$3.2 \times 10^3$</td>
</tr>
<tr>
<td>8</td>
<td>Extract + am123p</td>
<td>$4.0 \times 10^{10}$</td>
</tr>
<tr>
<td>9</td>
<td>Extract + am25p</td>
<td>$7.2 \times 10^7$</td>
</tr>
<tr>
<td>10</td>
<td>Extract + am28p + am25p</td>
<td>$1.2 \times 10^9$</td>
</tr>
</tbody>
</table>

Western blot analysis of HTLp-deficient, non-functional heads

We probed a nitrocellulose blot of wild-type and mutant phage proteins with adsorbed and unadsorbed serum (see Methods) to test the specificity of our adsorbed serum and confirm that the putative HTLp was indeed absent from defective heads and corresponded to band 11. The quantity of sample used was such that band 11 was not detected by Coomassie Brilliant Blue staining (Fig. 2b). The Western blot of these samples, using adsorbed serum as the first antibody, clearly displayed band 11 in the wild-type and am123p samples, but not in those of the inactive heads, am25p and am158p (Fig. 2c). The adsorbed serum however, revealed two other minor head proteins, corresponding to band 7 Mr 43000 and band 10 Mr 19000, in all four samples. Although the role of these proteins is unknown, they may be internal proteins and thus unavailable for removal of the corresponding antibodies during adsorption.

Conversion of non-functional to functional heads using a source of HTLp

We considered that cells infected with wild-type phage might contain HTLp in a free form just before lysis. Accordingly, an extract of such cells was treated with u.v. to inactivate the phage (see Methods) and was added to non-functional heads, am25p, for 60 min at 34 °C. The results (Table 2) show that although addition of excess functional tails (am28p) to the non-functional heads (am25p) made little difference to the titre of infectious phage (sample 5), the addition of u.v.-irradiated extract of wild-type phage-infected cells to the non-functional heads increased the titre by more than 100-fold (sample 9). This was not as large an increase in titre as that obtained by mixing functional heads with functional tails (sample 6) or functional heads with extracts of wild-type-infected cells (sample 8). This presumably reflects either a molar ratio of free HTLp to other components of less than one or the inefficiency of reassociation of HTLp with pre-formed heads. The extract of wild-type-infected cells did not complement am28p (sample 7) which confirms that it contained no active heads but a large quantity of functional tails in addition to HTLp. It would seem, therefore, that HTLp can be added to otherwise complete non-functional heads to restore their ability to combine with functional tails.

DISCUSSION

All the double-stranded DNA-containing bacteriophages which have been examined in detail possess heads of regular or prolate icosahedral shape. One of the vertices of the head carries the complex tail structure. The icosahedral head capsid has a fivefold symmetry at the vertex while the tubular tail has a sixfold rotational symmetry. This difference in symmetry was at one time viewed as an architectural paradox (Moody, 1965) but is now partially explained by the structure of the proteins found at the vertex. In the case of coliphages lambda, T4, P22, T3 and T7 and the Bacillus subtilis phage φ29, the vertex is formed by an oligomer composed of one or, in some cases, several of the minor structural components of the head. In the case of lambda, T4 and φ29, this oligomer has a 12-fold rotational symmetry (Kochan et al., 1984; Driedonks et al., 1981; Carrascosa et al., 1985; for review, see Bazinet & King, 1985). This protein structure has also been implicated in the initiation of prohead assembly (Murialdo & Becker, 1978) and in the
packaging and release of the phage DNA (Earnshaw & Casjens, 1980; Bazinet & King, 1985) and has therefore variously been named the initiator, the connector and the DNA-translocating vertex.

In the case of phage T5, less is known about the functions of the structural proteins. Zweig & Cummings (1973) and Heller (1984) found at least 15 structural proteins by SDS–PAGE of which the functions of five are known. Protein band 1 corresponds to the LTF (Saigo, 1978), band 2 to the STF (Heller & Schwarz, 1985), band 5 to the receptor-binding protein (Heller & Schwarz, 1985), band 6 to the major tail protein and band 8 to the major head protein (Zweig & Cummings, 1973). Our findings show that the HTLp (band 11) is essential for the attachment of the formed tail to the filled head to give active phage.

Our studies show that whereas the product of infection of non-permissive strains by a head-producing, tail-deficient amber mutant such as am123 can combine with functional tails produced by a second amber mutant, am28, the corresponding product of group 11 mutants, am25 and am158, although indistinguishable from functional heads on sucrose gradients and by electron microscopy, cannot complement with am28 tails.

Two possible explanations were considered: first, am25 heads may be deficient in some factor essential for the attachment of tails; second, such attachment may occur but the resulting particles are blocked in some function necessary for infection. We showed that whereas functional heads (am123p) and functional tails (am28p) could combine to yield particles which banded on velocity sedimentation gradients at a position typical of complete wild-type phage particles, non-functional heads (am25p) could not do so. This result eliminates the second possibility and confirms the first.

We have named the protein involved in this effect HTLp (head–tail linker protein). Its structure must differ from the vertex proteins of lambda (monomer $M_r$ 62 000) and T4 (monomer $M_r$ 65 000) since it has a monomer of $M_r$, 18 000. Zweig & Cummings (1973) estimated that it amounted to 1% of the phage protein, which suggests a copy number of four to five per virion. Another important difference between T5 HTLp and the vertex proteins reviewed above is that it does not appear necessary for DNA packaging, since the non-functional, HTLp-deficient heads contain the full complement of DNA as determined by restriction analysis (data not shown). One would therefore expect that one of the other T5 head proteins has the function of DNA translocation, and this may be an internal head protein such as that of band 7, $M_r$ 43 000, but it cannot be the protein of band 10 since that one is not essential (Saigo, 1978).

Since the yields of the products am28p, am25p and am123p from the non-permissive host appeared to be similar to that of wild-type phage, as judged by the heights of u.v.-absorbing peaks on the preparative gradients and the titres of active phage obtained from successful in vitro complementation, we conclude that the products of the genes defined by the corresponding mutations are not necessary for cellular lysis.

Since we observed that extracts of T5-infected cells prepared late in the latent period can bring about the ability of non-functional heads to combine with tails and produce active phage, we conclude, first, that an excess of HTLp over DNA-filled heads is produced during normal infection, and second, that the sequence of events during T5 maturation includes the addition of HTLp to the filled heads, followed by the attachment of tails to generate the complete phage.

Since HTLp-deficient heads are as stable as HTLp-containing heads (data not shown), we conclude that HTLp is not necessary for the integrity of the filled head.

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REFERENCES


Head–tail linker protein of T5


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