Volume 255, number 2, 435-440 FEB 07650 September 1989

Irreversible binding of bacteriophage T5 to its FhuA receptor protein is associated with covalent cross-linking of 3 copies of tail protein pb4

Andrea Feucht, Gerrit Heinzelmann* and Knut J. Heller

Universität Konstanz, Fakultät für Biologie, Konstanz and **Universität Tübingen, Institut für Biologie, Lehrstuhl für Mikrobiologie II, Tübingen, FRG*

Received 13 July 1989

Irreversible binding of bacteriophage T5 to its FhuA receptor protein is characterized by a high activation energy, typical for reactions where covalent bonds are formed [Zarnitz, M.L. and Weidel, W. (1963) Z. Naturforsch. 18b, 276–280]. Upon binding of radiolabeled T5 phages to FhuA formation of a new protein of 250 kDa was observed. Using electrophoretical and Western blotting techniques this protein was shown to be formed by cross-linking of 3 copies of tail protein pb4, rather than by cross-linking of FhuA and the receptor-binding protein.

FhuA receptor protein; Tail protein pb4; Western cross blot; (Bacteriophage T5)

1. INTRODUCTION

When Zarnitz and Weidel [1] studied the thermodynamics of the interaction between phage T5 and receptor particles from *Escherichia coli* B they calculated an activation energy of 11 kcal/mol for irreversible attachment of the tail tip to the receptor. The high value of activation energy needed made them believe that irreversible binding was achieved by formation of covalent bonds between the receptor and the phage.

Binding to the receptor of T5 is mediated by a minor tail protein (pb5) of 67 kDa [2,3]. This protein is not located at the very tip of the tail but at the distal end of the conical part, which marks the transition from the tail tube to the central straight tail fiber [3]. If the hypothesis of Zarnitz and Weidel were correct, one should be able to identify a protein of at least 145 kDa (67 kDa from pb5 + 78 kDa from FhuA) upon irreversible binding of T5 to its receptor. However, depending on the number of copies covalently linked, 145 kDa is the lowest estimate for the putative high molecular mass protein.

In this study, we show that a high molecular mass protein of 250 kDa is formed upon binding of T5 or T5 tails to FhuA. However, our results demonstrate that this protein is not formed by covalent cross-linking of FhuA and pb5 but rather by cross-linking of 3 copies of pb4. pb4 is an as yet functionally uncharacterized minor tail protein of 75 kDa [2]. Its location within the T5 tail is not known.

Correspondence address: K.J. Heller, Universität Konstanz, Fakultät für Biologie, Postfach 5560, D-7750 Konstanz, FRG

2. MATERIALS AND METHODS

2.1. *Bacteria, phages, and culture conditions*

For propagation of wild-type T5 *E. coli* F [4] and for propagation of amber phages *E. coli* F*su*β^+ [5] was used. *E. coli* LF930(pFB103/1) is a K-12 strain bearing a multicopy plasmid encoding the FhuA receptor protein [6]. *E. coli* CR63 is a wild-type (*fhuA*$^+$) K-12 strain.

The wild-type T5 used was T5st(H) [2]. For production of tails T5st*am*N5 was used [7].

Bacteria were grown in tryptone-yeast extract medium as previously described [8]. For phage propagation the medium was supplemented with 1 mM $CaCl_2$.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

2.2. *Isolation, purification and radiolabeling of T5 phages and tails*

Phages and phage tails were radiolabeled and purified as described in [7,9].

2.3. *Radiolabeling and purification of outer membranes*

E. coli LF930(pFB103/1) was grown for 3 generations in the presence of 5 μCi/ml [^{14}C]casamino acids. Outer membranes were prepared as described in [10].

2.4. *SDS-PAGE and autoradiography*

The system of Laemmli [11] was applied. Usually 11% acrylamide gels were used. However, for analyses of high molecular weight proteins, the acrylamide concentration was reduced to 8%, without changing the acrylamide/bisacrylamide ratio. For autoradiography gels were dried on filter paper.

2.5. *Western blotting*

Western blotting was performed as previously described [9], using protein A-coupled peroxidase and the peroxidase substrate 3-amino-9-ethylcarbazol for detection of antibodies.

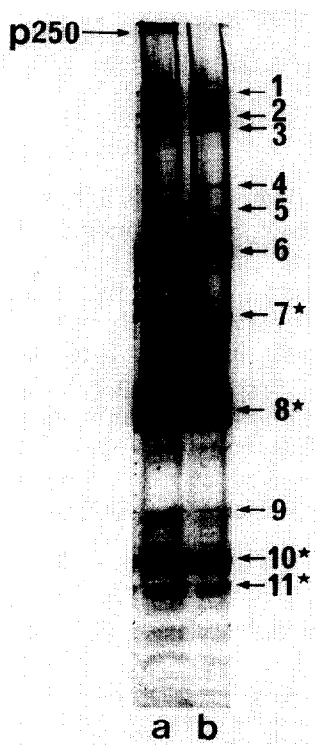

Fig.1. Binding of radiolabeled T5 phages to outer membranes. ^{14}C-labeled T5 phages were either incubated with outer membranes from *E. coli* F (lane a) or alone (lane b) for 30 min at 37°C and subsequently subjected to SDS-PAGE. An autoradiography of the dried gel is shown. Numbers to the right indicate phage proteins [2]. Tail proteins are indicated by numbers, head proteins by numbers with asterisks. The arrow to the left, labeled p250, shows the position of the newly formed protein in lane a.

3. RESULTS

Upon incubation of isolated outer membranes of *E. coli* CR63 with radiolabeled T5 phages, a radiolabeled protein was detected which was not seen in the radiolabeled phages alone (fig.1). The protein had an M_r of 250 000; it was therefore called p250.

Formation of p250 was also observed upon incubation of purified FhuA protein [12] with purified T5 tails (fig.2). The conditions were set that more than 95% of the tails were bound to the FhuA receptor particles at the highest FhuA concentration used (determined by electron microscopy (data not shown)). Fig.2 also shows that decreasing concentrations of FhuA resulted in decreasing formation of p250. Furthermore, increasing amounts of p250 were accompanied with decreasing amounts of tail protein pb4, suggesting participation of pb4 in the formation of p250.

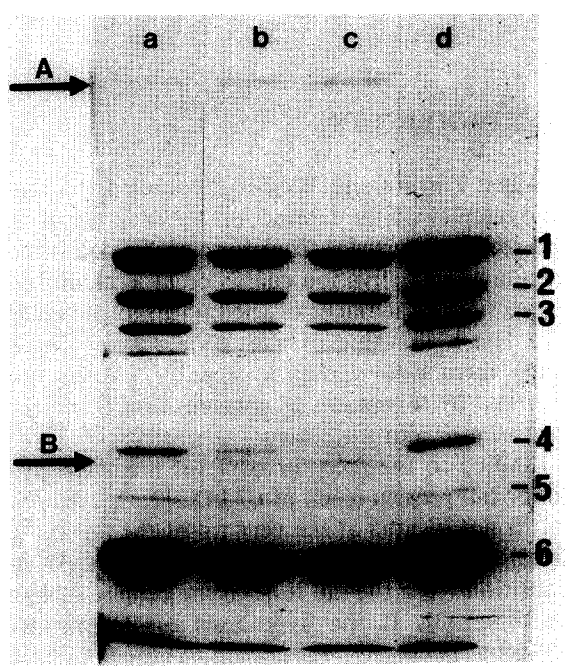

Fig.2. Binding of T5 tails to FhuA receptor protein. Tails and FhuA protein were incubated together for 30 min at 22°C and subsequently subjected to SDS-PAGE. Numbers to the right mark tail proteins. Arrow (A) indicates the position of p250, arrow (B) indicates the position of FhuA. Lane a: tails and FhuA in a 1:0.2 ratio; lane b: tails and FhuA in a 1:1 ratio; lane c: tails and FhuA in a 1:1.5 ratio; lane d: tails without FhuA. The amounts of FhuA applied to the gel in lanes a and d were about twice those in lanes b and c.

Though not entirely evident from fig.2, other experiments (not shown) indicated that the amount of input FhuA receptor protein was not reduced upon formation of p250. It should be noted that the amount of p250 in relation to tail proteins was usually rather low. The results shown in fig.2 were exceptional. They were obtained with tails used immediately after preparation. Only under these conditions p250 was formed in such amounts that a decrease in pb4 became evident.

To further test for a direct participation of FhuA in cross-linking 2 different approaches were used. First, T5 tails were incubated with radiolabeled outer membranes prepared from a strain overproducing FhuA [6]. After subsequent SDS-PAGE, formation of p250 was visible on the Coomassie blue-stained gel (fig.3A) but not on an autoradiography of the same track (fig.3B).

The second approach was to detect p250 in Western blots using antiserum raised against FhuA. Fig.4A shows that p250 could be detected in Western blots when anti-T5 antiserum was used. No protein corresponding to p250 could be detected with anti-FhuA antiserum (fig.4B).

The results so far demonstrated that p250 was not formed by covalent cross-linking of the receptor-binding protein of phage T5 to áhuA but rather by covalent cross-linking of phage proteins, at least one of which should be pb4. In order to test whether p250 was formed exclusively from pb4 or from different protein species, we applied a newly developed Western blot technique. This technique, which we call 'Western cross blot', will be described in detail elsewhere. However, the main features of this technique are as follows. Two protein samples, each applied to a different SDS-gel over the whole width of the gel, are electrophoresed and subsequently prepared for Western blotting up to the stage where antibodies are added to the blot. A polyclonal antiserum raised against one protein sample is added to one of the blots, called the first blot. After incubation surplus antibodies are

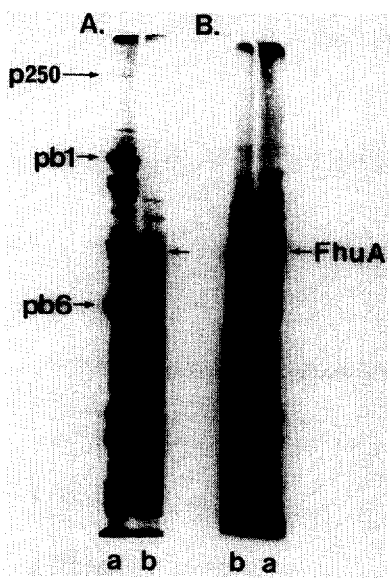

Fig.3. Binding of T5 tails to radiolabeled outer membranes from a FhuA-overproducing *E. coli* strain. Purified T5 tails were incubated with outer membranes from *E. coli* LF930(pFB103/1) for 30 min at 37°C and subsequently SDS-PAGE. The gel was stained with Coomassie blue (A), dried and autoradiographed (B). Lane a: outer membranes + T5 tails; lane b: outer membranes alone. The arrows to the right mark the position of the FhuA receptor protein. The arrows to the left mark the position of T5 tail proteins pb1 and 6 and of the newly formed protein p250.

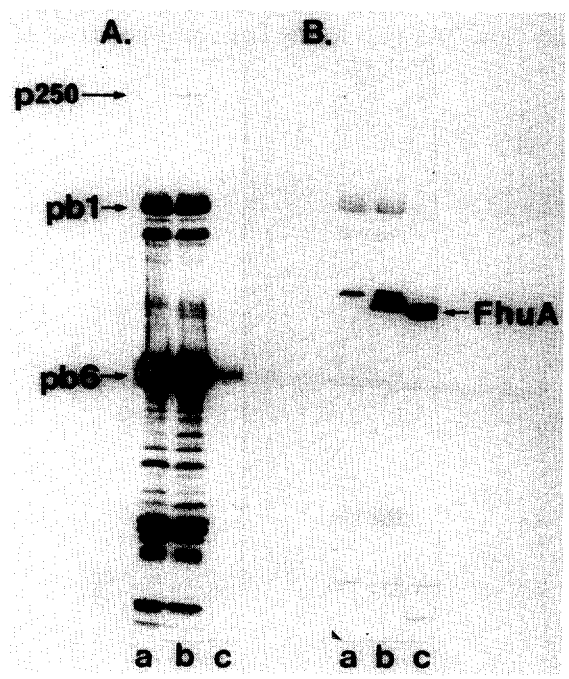

Fig.4. Western blots of T5 tails incubated with FhuA receptor protein. Two identical Western blots were prepared from T5 tails (lanes a), T5 tails + FhuA (lanes b), and FhuA (lanes c). In (A) anti-T5 antibodies were used to detect T5 proteins, while in (B) anti-FhuA antibodies were used. The arrows mark the positions of tail proteins, p250, and FhuA, respectively.

washed off. Then the second blot is placed face to face in a right angle onto the first blot, and both are incubated together with the pH lowered to 3. Under these conditions a fraction of the antibodies is released from their antigens on the first blot. These antibodies can bind to the second blot, provided a proper antigen is present on the blot where the antibodies were released. If two identical protein samples have been used, such places will be the cross-points of identical protein bands, and, in addition, cross-points between immunologically related proteins. In the case of p250 this means that antibodies released from p250 should bind to the protein species of which p250 is a component, or, the other way round, antibodies released from the protein species of which p250 is a component should bind to p250.

Fig.5 shows the second blots of two cross blots. In fig.5A the tails used for the first blot contained p250, while those for the second blot did not. In fig.5B both blots were done with tails containing p250. In the latter figure 2 stained spots are visible along the line where p250 was present on the second blot. The spot to the left corresponds to the cross-point with p250 from the first blot and the spot to the right corresponds to the cross-point with pb4 from the first blot. This indicates that p250 is immunologically related only to pb4 and to no other T5 protein from pb1 through pb6. This is in agreement with the fact that only the line of the second blot, corresponding to pb4, shows a stained spot at the cross-point with p250 from the first blot. In fig.5A the 2 upper stained spots are missing because no p250 was present on the second blot. It should be noted that, due to the very faint reaction seen with p250, the cross blots had to be heavily overdeveloped, which resulted in very high staining of the background. Taken together, the results clearly show that p250 is formed exclusively from pb4 as far as tail proteins pb1 through pb6 are concerned.

Whether tail proteins of lower molecular weight are involved cannot be ruled out. However, the results shown in fig.2 suggest that the decrease in the amount of pb4 is quantitatively identical to the increase in the amount of p250. This makes an involvement of other proteins unlikely, except for very small ones.

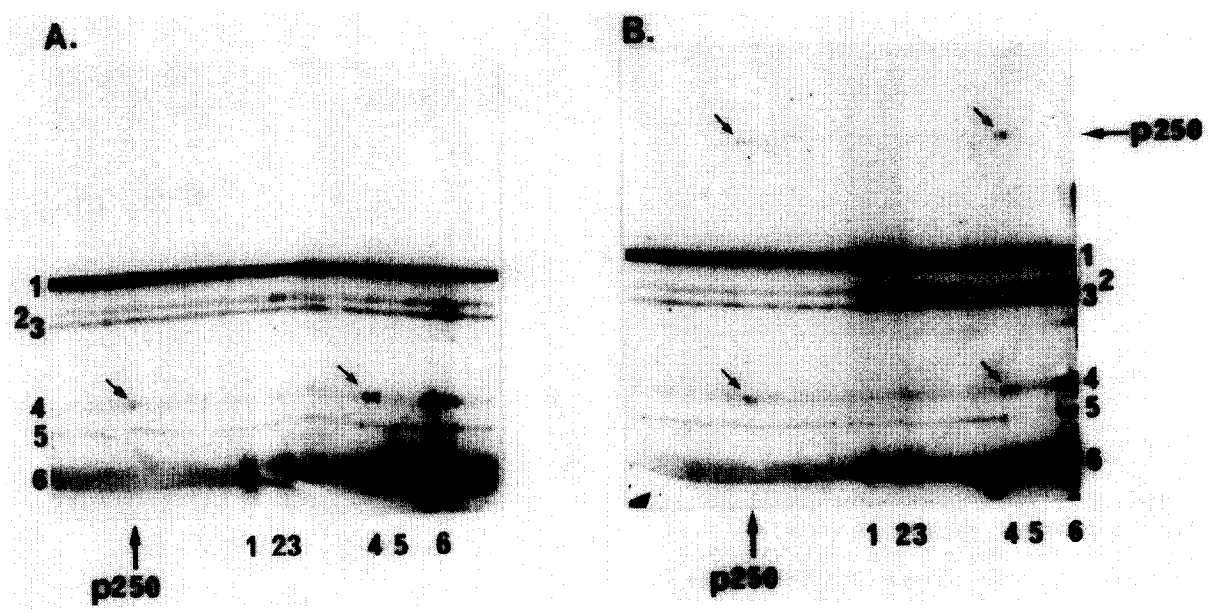

Fig.5. Western cross blots of T5 tails versus T5 tails + FhuA (A) and of T5 tails + FhuA versus T5 tails + FhuA (B). Blots were performed as described in the text. Numbers and the arrows marked p250 below the blots show the positions of the respective proteins on the first blot. Numbers at both sides and the arrow marked p250 on the right show the positions of respective proteins on the second blot. Small arrows within the figures show the cross-points between pb4 and p250 either with themselves or with each other.

4. DISCUSSION

In contrast to bacteriophages with contractile tail sheaths phages like T5, without contractile elements, do not show any obvious alteration of their morphology upon irreversible binding to the receptor. However, it is obvious that alterations within the phage particle upon binding to the receptor have to occur, albeit at a level not detectable in the electron microscope. One example shall explain the situation with T5: in the mature phage particle the DNA is connected to the proximal end of the tail [13]. Upon binding to the receptor a signal has to be carried from the receptor-binding protein along the tail to the head-tail connection to induce DNA penetration through the tail tube. Signal transmission may occur as a progressive conformational change of the major tail protein starting at the distal end of the tail tube. This would be similar to the described wave of conformational changes through the T4 tail tube, which is followed by contraction of the tail sheath [14].

There are examples for conformational changes of tail proteins induced by binding of non-contractile phages to their receptors [15]. However, the significance of these changes remains unclear. The same holds true for the observed cross-linking of pb4, as presented in this communication. Neither is the function of pb4 nor its localization within the tail known. There is, however, one indication that pb4 may be located at the distal end of the T5 tail, probably within the conical part. This indication derives from the assembly of the T5 tail. Deficiency of any of the tail proteins pb2, pb3, pb4, and pb6 (due to nonsense mutations within the respective genes) inhibits assembly of any tail-related structure (D. Bryniok and K.J. Heller, unpubl. results). This appears to be similar to what is known for phage lambda, where tail assembly starts with an 'initiator complex' comprising nearly all different tail proteins. Onto this 'initiator complex' the tail tube is subsequently assembled and finally tail assembly is finished by the addition of proteins to the proximal end of the tail tube [16].

If pb4 were located at the proximal end of the tail tube, one would expect to see in the electron microscope almost normal looking tails in pb4$^-$ lysates. This is not the case. Also, a similar location (and function) like the 'tape measure' protein (gpH) of lambda [17] seems unlikely, since such a protein can hardly be imagined to possess enzymatic activities (as will be discussed below). The most likely location of pb4, therefore, appears to be the conical part of the tail, which may be regarded as the 'base-plate' of T5.

What could be the function of pb4 located within the base-plate of T5? We think that it stabilizes a conformation of the base-plate, which is compatible with the penetration of DNA through the base-plate. The conformational change, induced by binding to the receptor, has to be transmitted to the major tail protein to result in a conformational change of the whole tail tube, also compatible with penetration of DNA. However, chaning the conformation of the whole tail tube in a progressive way may generate an increasing structural resistance and may eventually result in a flip-back to the original conformation followed by release of the phage from the receptor. Covalent cross-linking of pb4 could be the event which renders the conformational change of the base-plate irreversible and, therefore, makes a flip-back impossible.

The mechanism by which cross-linking of pb4 occurs remains unknown. We favour the idea that pb4 has a transpeptidase activity, responsible for cross-linking. We are currently engaged in characterizing pb4 as well as the D17 gene encoding pb4 in order to gain insight into the possible enzymatic activity of this structural protein.

Acknowledgements: We thank V. Braun and H. Hoffmann for the gift of purified FhuA. This work was supported by the Deutsche Forschungsgemeinschaft (He 1375/3-1 and -2).

REFERENCES

[1] Zarnitz, M.L. and Weidel, W. (1963) Z. Naturforsch. 18b, 276–280.
[2] Heller, K.J. (1984) Virology 139, 11–21.
[3] Heller, K.J. and Schwarz, H. (1985) J. Bacteriol. 162, 621–625.
[4] Lanni, Y.T. (1958) Virology 5, 481–501.
[5] Hendrickson, H.E. and McCorquodale, D.J. (1971) J. Virol. 7, 612–618.
[6] Fecker, L. and Braun, V. (1983) J. Bacteriol. 156, 1301–1314.
[7] Zweig, M. and Cummings, D.J. (1973) Virology 51, 443–453.
[8] Heller, K.J. and Braun, V. (1989) J. Bacteriol. 139, 32–38.
[9] Heller, K.J. and Bryniok, D. (1984) J. Virol. 49, 20–25.
[10] Osborn, M.J., Gander, J.E., Parisi, E. and Carson, J. (1972) J. Biol. Chem. 247, 3962–3972.
[11] Laemmli, U.K. (1970) Nature 227, 680–685.

[12] Hoffmann, H., Fischer, E., Kraut, H. and Braun, V. (1986) J. Bacteriol. 166, 404–411.
[13] Saigo, K. (1975) Virology 68, 154–165.
[14] Eiserling, F.A. (1983) in: Structure of the T4 virion (Mathews, C.K. et al., eds) Bacteriophage T4, pp. 11–24, ASM, Washington, DC.
[15] Roessner, C.A. and Ihler, G.M. (1984) J. Bacteriol. 157, 165–170.
[16] Katsura, I. (1983) in: Tail Assembly and Injection (Hendrix, R.W. et al., eds) Lambda II, pp. 331–346, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[17] Katsura, I. and Hendrix, R.W. (1984) Cell 39, 691–698.