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Localization of Minor Protein Components of the Head of Bacteriophage T4

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The bacteriophage T4 capsid contains a number of minor proteins that are required for head assembly but whose detailed function and position in the head are unknown. We have found that by systematically varying the conditions of extraction, some of these minor proteins can be removed while the main capsid structure is left substantially intact. Electron microscopic examination of the residual capsids showed that the extraction of the product of gene 20 is correlated with the loss of a plug that distinguishes one vertex position (presumably the tail attachment site) from the others. Extraction of the product of gene 24 is correlated with the loss of the other 11 (nonproximal) vertexes of the capsid. We further show that antibody to P24 binds specifically to the nonproximal vertexes of both T4 preheads and T4 phages. On the basis of our findings, we suggest that P20 is located at or near the tail attachment site of the capsid, whereas P24 forms the 11 nonproximal vertexes of preheads and P24* forms the nonproximal vertexes of the mature head.

The head of bacteriophage T4 consists of an elongated icosahedral shell (2, 8, 24), or capsid, which encloses the DNA, internal proteins, and internal peptides. Purified heads have been reported to contain at least 8 to 11 protein species (10, 20) and, possibly, as many as 22 (D. Coombs and F. Eiserling, personal communication). Of these, at least 4 are internal proteins (6, 10, 14, 15, 33), and 6 are reported to be a part of the "neck," or head-tail junction (Coombs and Eiserling, in press). Many of the remainder have not been identified with known T4 genes, and their functions are unknown.

The five principal proteins of the T4 capsid are P20, P23*, P24* (P stands for protein product of the gene whose number follows, and * denotes the proteolytically processed form of the gene product), *hoc*, and *soc* (13, 16, 20). Each of these has been demonstrated to be either quantitatively important or essential for capsid formation, or both. Studies of giant variants of T4 and T2 by optical diffraction and reconstruction of filtered images of electron micrographs show that the elongated part of the T4 capsid has a (6+6+1)-type morphological subunit (3, 16). Comparisons of the protein composition and filtered micrographs of T4 and T2, as well as T4 mutant, phages suggest that this morphological subunit is composed of a hexamer of P23* with a molecule of *hoc* at its center, adjacent hexamers being bridged by trimers of the *soc* protein (16).

This model is supported by analogous studies on the capsoid shells of polyheads. These consist of hexagonal arrays of P23 (the uncleaved form of the major capsid protein) folded into cylinders of varying diameter (12, 19, 31, 35). If the P23 of these polyheads is cleaved in vitro, *hoc* and *soc* are taken up by the P23* lattice. The new lattice is indistinguishable, except for pitch, from the lattice of T4 giant phages. The resulting polyheads contain only the proteins P23*, *hoc*, and *soc* (32). These results show that the elongated part of the T4 capsid need not contain P24* or P20. The location and function of these two capsid proteins have not been established. P24 is cleaved from a molecular weight of 47,500 to P24*, with a molecular weight of 45,000, during capsid maturation (20). There are between 50 and 150 copies of P24* per capsid (2; L. Onorato, unpublished observations). P24 is assumed to affect the capsid structure since mutants that confer osmotic shock resistance to the phage are located in gene 24 (unpublished results, quoted in reference 23). Bijlenga et al. (3) have suggested that P24* might form one of the two icosahedral caps of the capsid, since its quantity does not increase proportionately to P23* in giant phage. However, it is clearly not required for cap formation, since amber as well as temperature-sensitive mutants in gene 24 form closed "τ-particles" (11, 18, 22). It has been shown that 24*ts* τ-particles can be converted to morphologically normal infectious phage after a shift to permissive temperature (5), indicating that they are

normal preheads whose development is arrested by the gene 24 mutation.

The product of gene 20 has a molecular weight of 64,000 (20). About 10 to 20 copies are found per phage capsid (2). Mutants in gene 20 produce only open-ended polyheads and no closed structures, suggesting that P20 is required for cap formation (18, 22).

Since there is no direct evidence for the position of either P20 or P24* on the T4 capsid, we have used chemical and immunological techniques to localize these proteins, in the hope that a knowledge of their location will clarify their roles in capsid formation.

MATERIALS AND METHODS

Bacteria. Bacteriophage stocks were grown and titrated on *Escherichia coli* CR63 (su^+). Defective lysates were prepared on *E. coli* B^e (su^-).

Bacteriophage. The wild-type strains used were T4D and T2L. Mutants used are derivatives of T4D and have been described (29) except for bypass 24 (Byp24). The latter was kindly provided by L. Black. It has recently been characterized as a cold-sensitive double mutant of genotype 23(*cs*)-23(*amN*65) (L. Black, L. McNicoll, and L. Simon, personal communication). Byp24 is propagated at its permissive temperature, 40.5°C.

Media. Stocks and defective lysates were grown in medium M9A, i.e., M9 supplemented with Casamino Acids at a final concentration of 1% (1).

Defective lysates. Defective lysates were prepared on *E. coli* B^e as previously described (24). Normally, the infected cells were concentrated 100-fold by centrifugation before lysis. When a lysate was prepared for absorbing antiserum, it was centrifuged for 10 min at 10,000 × *g* to remove infecting phages attached to cell debris before use. Lysates of Byp24 were made on *E. coli* B^e at 25°C.

Preparation of antigens. P24 was prepared as previously described (29) from a lysate in which its processing is prevented in vivo and in vitro by a mutation in gene 21.

Antisera. Anti-*hoc* was a gift of R. Bijlenga, who also kindly helped in the preparation of anti-P24. Both sera were obtained by injection of the purified antigens into the hind footpads of rabbits in Freund complete adjuvant as previously described (25). Ferritin-conjugated antibodies to rabbit immunoglobulin were obtained from Cappel Laboratories, Downingtown, Pa. Except as noted, anti-P24 serum used for decorating phages for examination by electron microscopy was routinely absorbed with an equal volume of 24 (*amN*65) defective lysate prepared as described above. The absorption was carried out at 4°C overnight, and the serum was clarified by centrifugation at 15,000 × *g* for 20 min before use.

Preparation of capsids and preheads. T4 phage heads were isolated from lysates of the double mutant in the tail genes 10(*amB*255)-18(*amE*18) by sucrose density gradient centrifugation (2). The preparation of 23(*tsA*78) aberrant preheads has been described (25). Aberrant preheads from mutants in gene 21 were isolated in a similar fashion, except that glycerol gradients were used. They were fixed with 2% formaldehyde, dialyzed, and stored at 4°C in 0.1 M KH_2PO_4, pH 7.0. A detailed description of these particles and their preparation will be published (L. Onorato, manuscript in preparation).

Differential extraction. Capsids were extracted in urea by dialysis overnight at 4°C against a buffer containing 7 M urea, 5.85 g of glycine per liter, and 7.5 g of NaCl per liter adjusted to the appropriate pH with NaOH. Residual capsids were separated from the extracted proteins by centrifugation for 3 h at 50,000 × *g*. The pellet was suspended in 0.06 M Tris-Cl, pH 7.4.

Capsids were extracted with sodium dodecyl sulfate (SDS) by suspending them in SDS electrophoresis sample buffer (20) followed by incubation at the specified temperature for 10 min in a water bath. Residual capsids were separated as described above.

Acrylamide gel electrophoresis. Slab gels containing 10% acrylamide and 0.1% SDS were run in the Studier modification of the apparatus described by Reid and Bieleski (27). The buffer system was that described by Laemmli (20). The slabs were stained for 30 min in 0.25% Coomassie brilliant blue in 50% methanol-10% acetic acid and destained overnight in 10% methanol-7% acetic acid. All samples were incubated 10 min in SDS sample buffer at 100°C before being applied to the gel.

Immunoreplicate electrophoresis. Anti-P24 serum was diluted with water and mixed at 50°C with 1.2% molten agarose to give a final concentration of 25% antiserum and 0.6% agarose. This was pipetted onto the surface of unstained, unfixed SDS slab gels, and the immune replica was developed, washed, and stained as described by Showe et al. (30).

Other immunological techniques. Ouchterlony plates were made with microscope slides as previously described (25). The conditions for decoration of preheads and capsids with antibody are described in the figure legends and in Results.

Identification of gene products. The identification of P24 and P24* on SDS gels was established with antiserum and 24^- amber mutants as described in Results. Since antiserum to P20 was not available, the position of the P20 band of SDS gels was established by comparing radioactive lysates of 20^+ and 20^- amber mutants as previously described (20). The internal protein PB1*, or *alt*, has been reported to migrate both faster (15, 26) and slower (9) than P20. To insure that we properly distinguished these two similarly migrating species, normal phages were compared with phages isolated from the *ac-q* mutant, which lacks PB1* (15). In addition, immune replicas were prepared with antiserum against PB1*. These tests showed PB1* to migrate slightly slower than P20 under our conditions and to be present in considerably larger amounts than P20 in purified heads.

Electron microscopy. Micrographs were made on a Philips 300 electron microscope operating at 80 kV with a liquid nitrogen anticontamination device.

Specimens were adsorbed to a carbon film as described by Aebi et al. (2) and negatively stained with 2% sodium phosphotungstate at pH 7.2. Micrographs were taken under conditions of restricted

irradiation (32), usually at a magnification of ×45,000 and an underfocusing of 500 to 800 nm to increase contrast, and recorded on Kodalith LR EM film.

RESULTS

Differential extraction of P20 and P24 from capsids. T4 capsids are resistant to both 1% SDS and 8 M urea at room temperature and neutral pH. By raising the temperature in SDS solution or the pH in urea solution, however, these structures can be dissolved (17, 20). We have varied these parameters to find conditions for differentially removing one or another of the minor proteins from the capsid.

After treatment at pH 11.0 in 7 M urea, we found that the capsids, as viewed by negative staining in the electron microscope, were still substantially intact. However, they lacked the fold characteristic of untreated capsids visualized in negatively stained preparations, and gaps or openings were seen at the vertexes (Fig. 1). When structures were removed from the urea solution by centrifugation and the extracted proteins were analyzed by SDS gel electrophoresis, it was found that, in addition

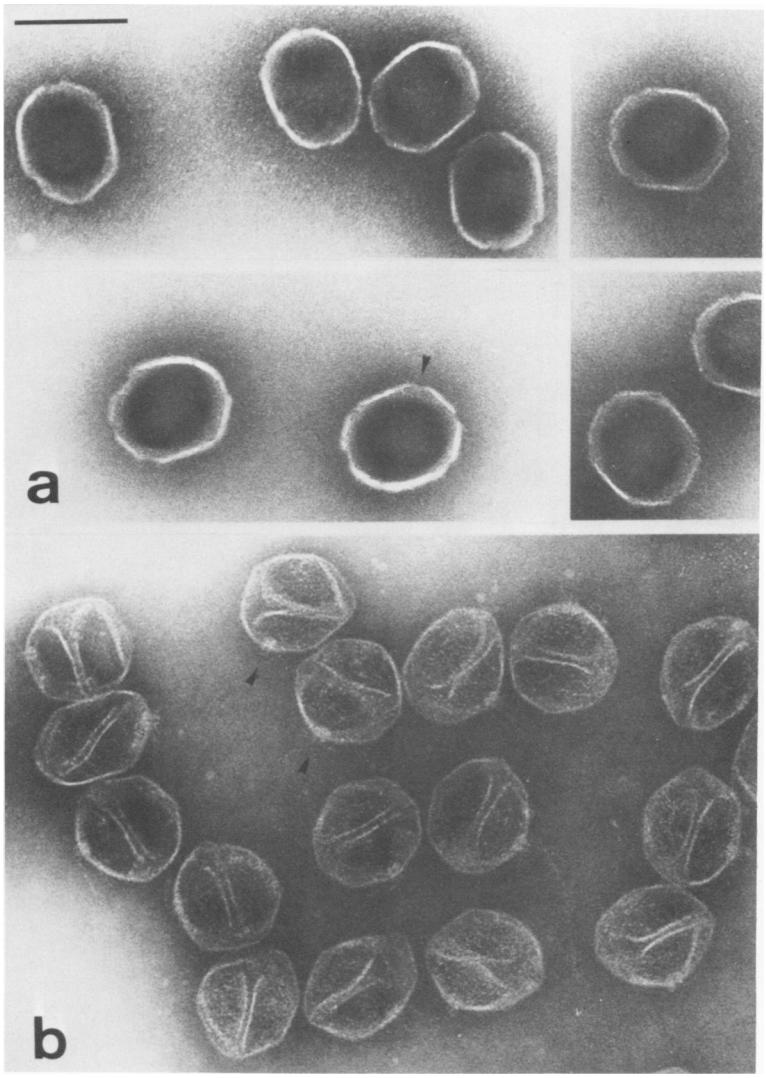

FIG. 1. *Micrographs of capsids of bacteriophage T4D after treatment in alkaline urea and of untreated capsids. (a) Capsids incubated in 7 M urea at pH 11.1. The arrow shows a gap at a vertex. These residual capsids were also treated with SDS at room temperature after removal of urea and thus have also lost most of their neck structure. (b) Untreated capsids. Arrows show the plug and fibrils at the neck of the capsid. Bar = 0.1 μm for all micrographs.*

to internal proteins, some of the capsid shell proteins, P23*, P24*, and *hoc*, had been extracted. To establish a correlation between the appearance of the gaps at capsid vertexes and the differential extraction of one or more gene products, capsid appearance and proteins extracted were compared for several series of varying conditions.

The effect of temperature variation during extraction by 1% SDS is shown in Fig. 2. At 55 or 65°C the capsids still appeared intact except for the plug and fibrillar material at their bases (cf. Fig. 1b with 2). The only proteins quantitatively removed at 55°C were P20, *wac*, IPIII, and Px, a protein with a molecular weight of 33,000 that we have not further identified. At 65°C, the last traces of PB1* were removed from the capsids, and some P23* was also solubilized. At 70°C the capsids underwent substantial disaggregation (Fig. 2c). Only P23* and some *hoc* now remained in the residual "capsids," all of the other proteins having been removed. Thus,

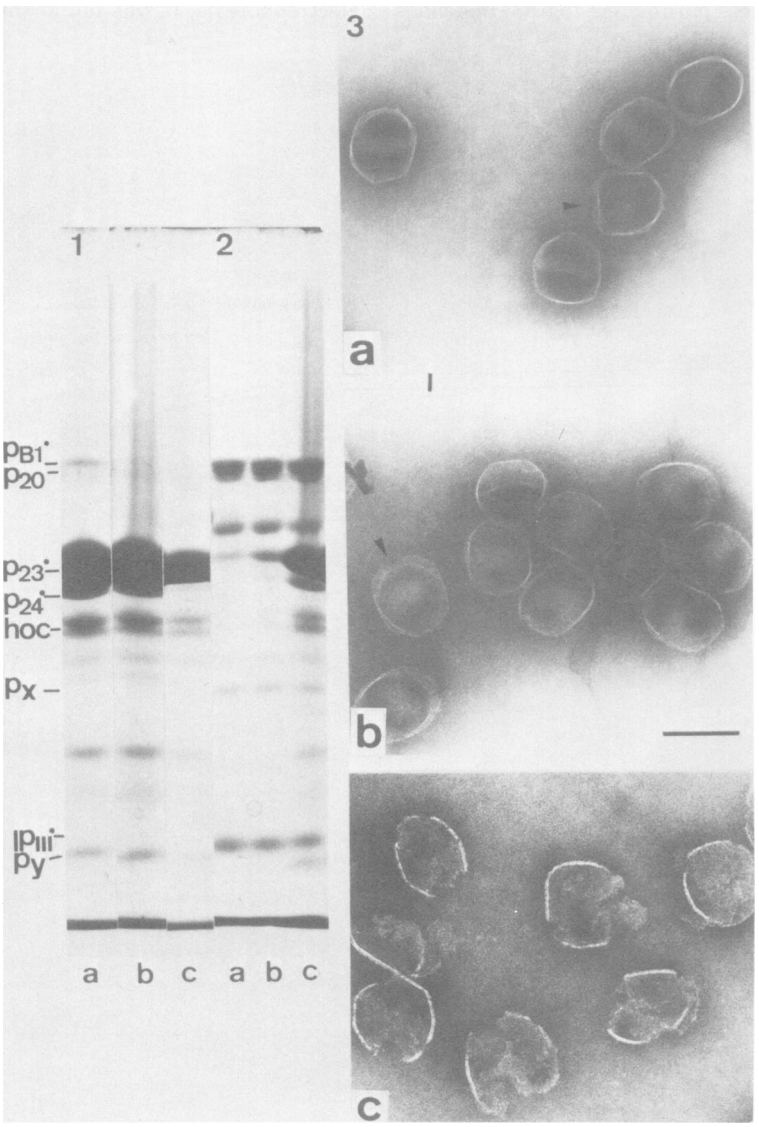

FIG. 2. *Differential extraction of T4 capsids in SDS sample buffer at various temperatures: (a) 55°C, (b) 65°C, and (c) 70°C. SDS gels (10%) of (1) the centrifugal pellet, containing the residual capsids, and (2) the supernatant, containing the extracted proteins; (3) micrographs of the residual capsids. Bar = 0.1 μm for all micrographs.*

the capsid structure appears to resist increasing temperature in SDS solution as a unit except for a few proteins. In particular, the vertexes are nearly as resistant as the rest of the capsid. P20 and Px are significantly less SDS resistant. They are solubilized at 55°C, a temperature also sufficient to remove any visible basal plug.

Figure 3 shows a series of extractions in 7 M urea at varying pH. At pH 10.7 the capsid appearance was unchanged. Only *wac* and the internal proteins IPIII and PB1* were extracted in appreciable amounts. At pH 10.9, the capsids lost their characteristic fold, and gaps were seen at some of the vertexes. An appreciable amount of P24* and P23* as well as some *hoc* had been removed in addition to the proteins extracted at pH 10.7. At pH 11.1 the capsids lost their angular appearance, and gaps or discontinuities were visible at many of the vertexes. Although still more P23* and *hoc* had been extracted, the striking result is that no P24* remained in the residual capsids, whereas very little P20 or Px had been extracted. A plug was still visible at the base of most of the residual capsids (see also Fig. 8e). These results suggest

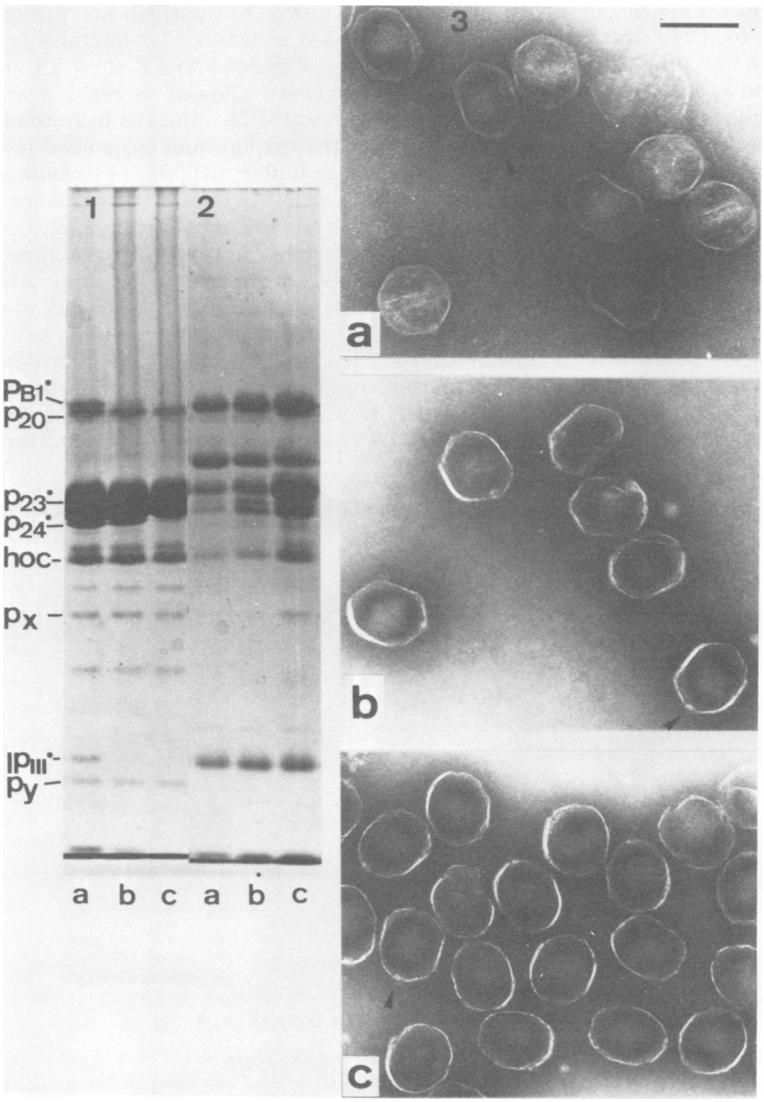

FIG. 3. *Differential extraction of T4 capsids in 7 M urea at various pH values: (a) pH 10.7, (b) pH 10.9, (c) pH 11.1. SDS gels (10%) of (1) the centrifugal pellet, containing the residual capsids, and (2) the supernatant, containing the extracted proteins; (3) micrographs of the residual capsids. Bar = 0.1 μm for all micrographs.*

that P24* is located at the capsid vertexes and that its extraction by urea at alkaline pH is responsible for the gaps in the residual capsids. The fact that P20 was not extracted when the vertexes had been removed suggests that it is located elsewhere.

Characterization of anti-P24 by immunoreplicate electrophoresis. To confirm the identity of P24* extracted from residual capsids, we used antiserum directed against P24 purified from a 21(*N*90)-23(*amH*11) defective lysate. Figure 4 shows an immune replicate made from a 10% SDS acrylamide slab gel with anti-P24 serum. Crude lysates from T4 head-gene mutants in which P24 is not cleaved showed a band corresponding to P24 (tracks e, i, and j), whereas amber mutants 24(*N*65) and 24(*B*26) lacked this band and had a new antigen with a molecular weight of 20,000 to 23,000 (tracks g and h). This new antigen may correspond to the amber fragment produced by these mutants, but we have not investigated it further.

Untreated capsids (track a) lacked the P24 antigenic species and had instead, as expected, an antigen of slightly higher mobility corresponding to the processed form of P24, P24*. This band was greatly diminished in the residual capsids (track c), even when a considerably greater amount was applied to the gel (compare the stained gel from which the replicate was taken). These results show (i) that the antiserum detects the protein identified genetically as the product of gene 24, (ii) that it also reacts with P24*, and (iii) that this antigen corresponds to one of the proteins removed from the residual capsids on treatment with 7 M urea at pH 11.1.

Track d is purified phage from the mutant Byp24 isolated by L. Black. These phage are reported not to contain P24 or P24* (Black et al., personal communication), and the immune replicate shows that they contain no corresponding antigen.

Localization of P24 antigen in phage. The antiserum to P24 characterized above was used to localize P24 antigen on capsids and capsid-related structures by immunoelectron microscopy. Figures 5 and 6 show T4 and T2 phages that were allowed to react overnight at 4°C with anti-P24, removed from the antiserum by centrifugation, and suspended in 0.05 M phosphate buffer, pH 7.0, for examination. Both of these closely related phages were seen to react with the serum. Characteristically, the phages were joined at their vertexes. Free particles had spikes at the vertexes, and where particles were agglutinated they could often be seen to be joined by a bridge between two vertexes. We show in control experiments described below that this material is immunoglobulin and that the vertex-to-vertex reaction is specific for anti-P24 serum. These results confirm the localiza-

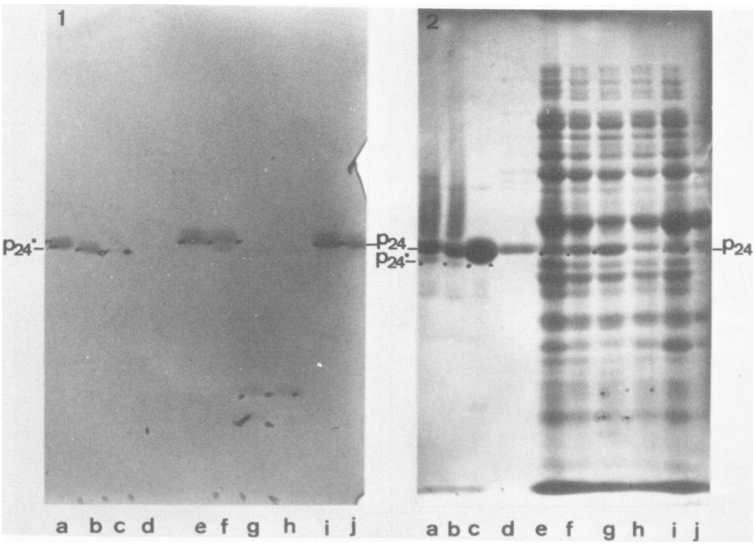

FIG. 4. *(1) Anti-P24 immunoreplicate from a 10% SDS polyacrylamide gel and (2) the corresponding gel stained after the replicate was removed, showing the mobility of P24 antigens in the applied samples. The samples were: residual capsids after extraction in 7 M urea at (a) pH 10.7, (b) pH 10.9, (c) pH 11.1; (d) purified phage particles from the mutant Byp24; defective lysates from mutants (e) 20(amN50), (f) 24(tsL90), (g) 24(amN65), (h) 24(amB26), (i) 21(tsN8), (j) 21(amE322). The black marks are needle holes, which serve to mark the antigen bands of the overlay on the SDS gel beneath.*

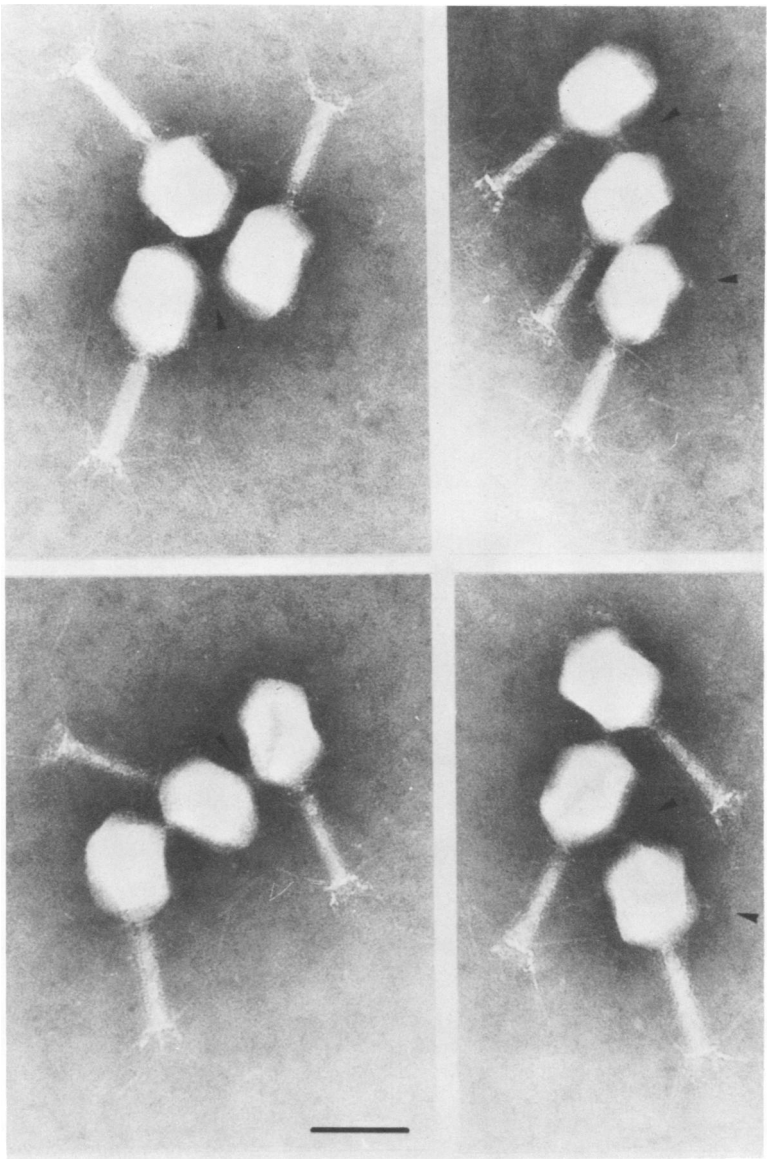

FIG. 5. *Micrograph of T4 phages reacted overnight with anti-P24. The serum was previously absorbed with a 24(amN65) lysate to remove possible contaminating antibody. Arrows indicate antibody bridges between vertexes of agglutinated phage and spikes on free vertexes. Bar = 0.1 μm for all micrographs.*

tion of P24* at the capsid vertexes suggested by the composition of residual capsids that lack vertexes and also P24*.

Localization of P24 antigen in aberrant preheads. The reaction of anti-P24 with capsids was weak. Apparently, few antibody molecules reacted at each vertex since the spikes were extremely difficult to visualize in the electron microscope. Previous studies (L. Onorato, Ph.D. thesis, University of Pennsylvania, Philadelphia, Pa.) suggested that preheads might react better with anti-P24, since 23*ts* aberrant preheads were found to be at least four times as efficient as capsids at absorbing anti-P24 serum. Figure 7d shows 23*ts* preheads decorated with anti-P24. As with capsids, bridges and spikes were seen on the particles. The reaction was both stronger and more rapid than it was with capsids, since the decoration was visible after a 30-min incubation (versus overnight, required for capsids).

The form of the 23*ts* preheads was too dis-

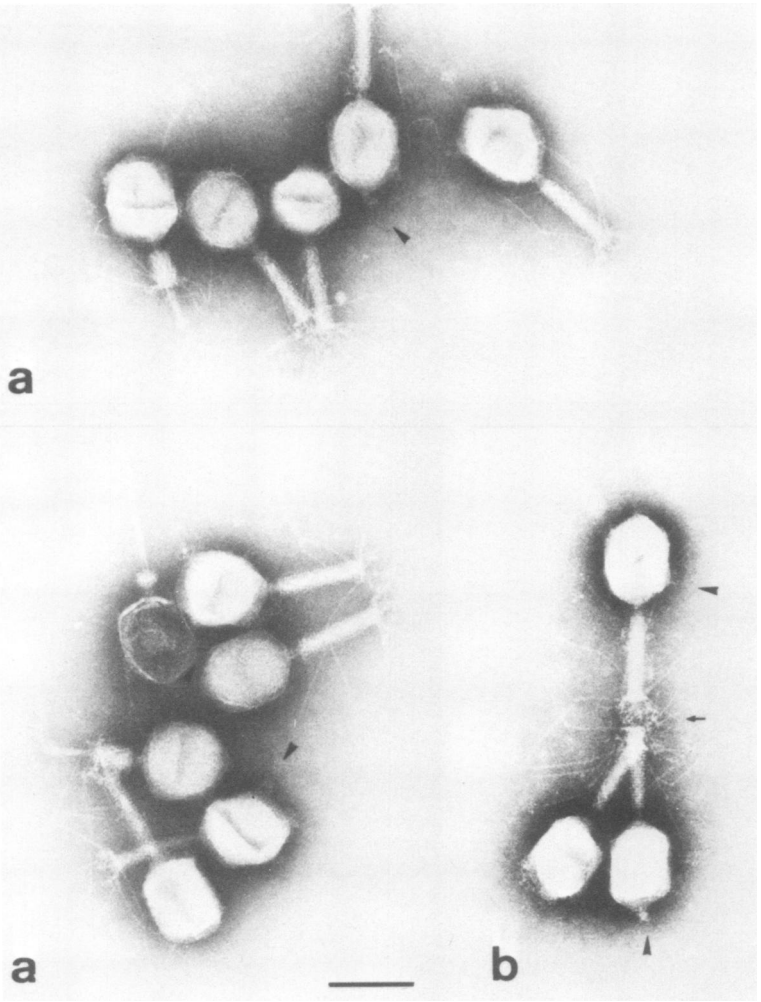

FIG. 6. *Micrographs of T2 phages reacted overnight with anti-P24. (a) Antiserum previously absorbed as in Fig. 5; (b) unabsorbed anti-P24. Arrow indicates contaminating antibody to phage baseplates removed by absorption; arrowheads indicate antibody spikes and bridges, as shown in Fig. 5. Bar = 0.1 μm for all micrographs.*

torted to permit the localization of the spikes relative to vertexes (Fig. 7d). Therefore, the preheads produced by mutants in gene 21 (*tsN8* and *amE322*) were treated with anti-P24. These particles are probably identical to normal preheads before cleavage since they lack only the T4 prehead proteinase activity, which is coded by gene 21 (30). Again, the reaction was both more rapid and stronger than that with capsids. On some particles nearly every vertex could be seen to be decorated (Fig. 7a, b, and e), unlike capsids, in which antibody could usually be seen on only one or two of the vertexes.

Antigenicity of P24 in partially extracted capsids. Preheads showed a strong reaction to anti-P24 after a 30-min exposure to antiserum, whereas capsids or phages gave only a weak reaction even after an 18-h exposure. This could result from a partial shielding of the P24* by P23* as a consequence of the lattice expansion (2) that accompanies prehead maturation. The fact that significant amounts of P23* were removed with the P24* during vertex extraction suggests that the remaining P24* in residual capsids might be more exposed than that in untreated capsids. We therefore compared the antigenicity of untreated capsids with those that had been extracted in 7 M urea at pH 10.9 and at 11.1. The untreated capsids and the capsids from which nearly all of the P24* had been removed by extraction at pH 11.1 showed no detectable agglutination after a 30-min incu-

bation with antiserum (Fig. 8b and d). However, the residual capsids extracted at pH 10.9, from which some P23* as well as some P24* had been removed, showed an increase in antigenicity. Like preheads, they agglutinated significantly after a 30-min exposure to anti-P24 (Fig. 8c).

Controls: dependence of vertex-to-vertex agglutination on P24 antigen and anti-P24. Capsids might tend to agglutinate by their vertexes even though the capsids themselves were uniformly covered with an antigen. *hoc* ought to have such a distribution, since there is thought to be a molecule of *hoc* at the center of each P23* hexamer (16). Figure 8a shows the appearance of capsids treated for 30 min with anti-*hoc* serum. They appear to be uniformly covered with antibody and show no special tendency to agglutinate vertex-to-vertex.

Figure 7c shows that the spikes and bridges between particles agglutinated with anti-P24 are composed of antibody. In this experiment anti-P24-agglutinated preheads were centrifuged, suspended in buffer, and then incubated with ferritin-conjugated goat anti-rabbit immunoglobulin. The spikes and bridges were seen to be labeled with the ferritin-conjugated goat antibody.

Immunoelectron microscopy can detect small amounts of antibody, and it is possible that the

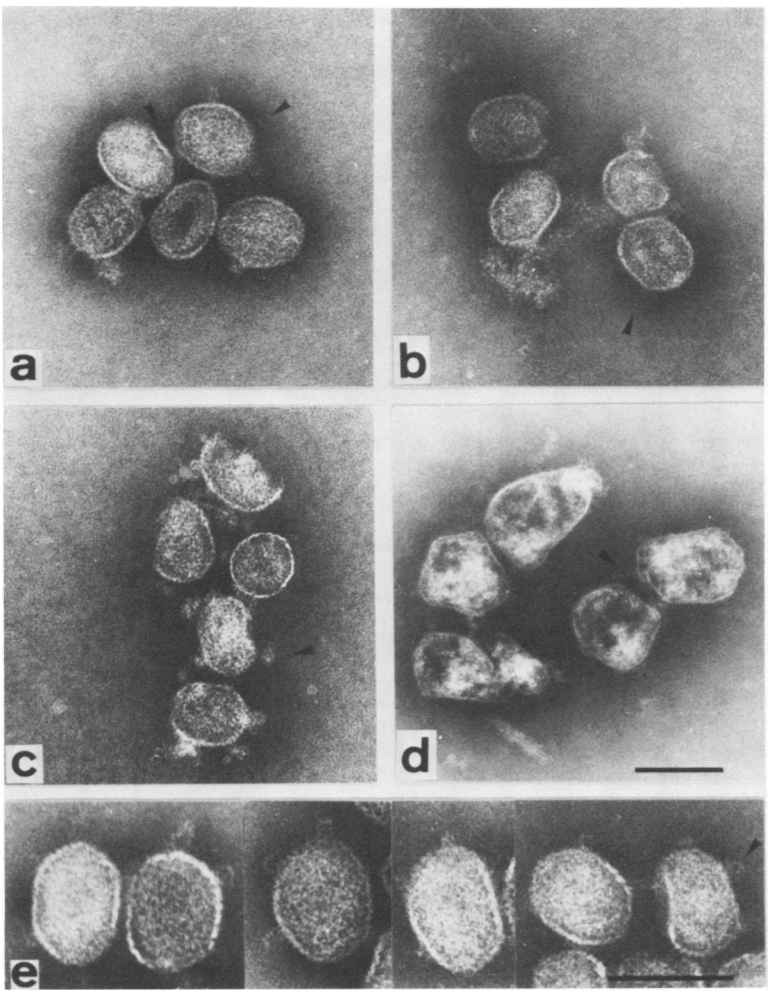

FIG. 7. *Micrographs of mutant preheads after a 30-min incubation with 24(amN65)-absorbed anti-P24. (a) Preheads from 21(tsN8); (b) preheads from 21(amE322); (c) 21(tsN8) preheads labeled with anti-P24 as in (a), then postlabeled with ferritin-conjugated goat anti-rabbit immunoglobulin. (d) "Crummy" preheads from 23(tsA78); (e) the same preparation as shown in (a) but at higher magnification. Arrows show antibody decoration at vertexes except (c), where it shows ferritin-conjugated antibody. Bar on (d) = 0.1 μm for (a), through (d), bar on (e) = 0.1 μm.*

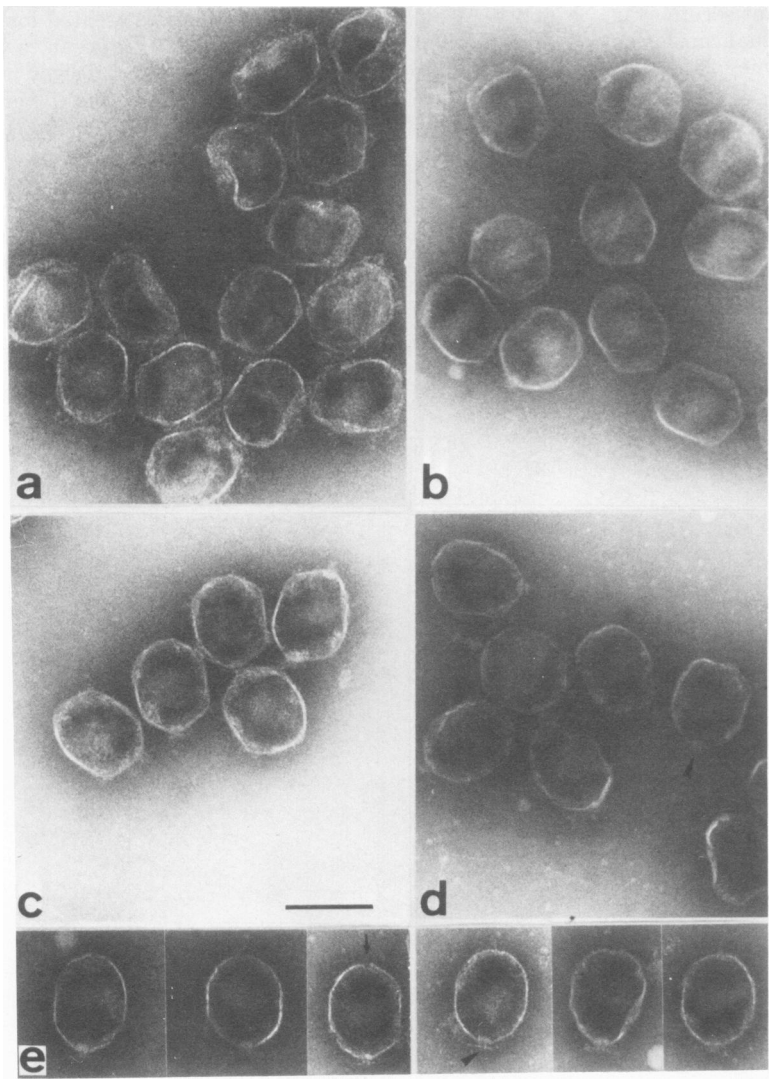

FIG. 8. *Micrographs of untreated and residual capsids after 30 min of incubation with anti-P24. (a) Untreated capsids incubated with anti-hoc; (b) untreated capsids incubated with anti-P24; (c) residual capsids extracted in 7 M urea, pH 10.9, incubated with preabsorbed anti-P24; (d) residual capsids extracted in 7 M urea, pH 11.1, incubated with preasorbed anti-P24; (e) same preparation shown in (d) except that particles are selected to show the fibrillar structures remaining at the neck even when gaps are present at the positions of the other vertexes. Arrow shows a distal vertex (gap); arrowheads show necks. Bar = 0.1 μm for all micrographs.*

vertex decoration that we observed might be due to contaminating antibody not detected by the immunoreplicate. The following experiments demonstrate that the vertex-to-vertex agglutination that we observed required both anti-P24 in the serum and P24 antigen in the particles.

Portions (100 μl) of antiserum absorbed with lysates from 23⁻ or 23–24 double amber mutants were compared for the loss of the P24 precipitin band on Ouchterlony plates and loss of agglutinating ability. Figure 9 shows the results. While 25 μl of 500× concentrated 23⁻ lysate resulted in the loss of ability to give a precipitin line, even 16 times more 23–24 lysate had no effect beyond dilution. When the absorbed sera were tested on preheads, 100 μl of 23⁻ lysate was found to have removed all capacity to agglutinate (Fig. 9c), whereas 400 μl of 23–24 lysate had no effect (Fig. 9b). Higher amounts

of 23-24 lysate could not be tested because the resulting dilution is too high to give a reaction even when buffer instead of serum is used as diluent. These results show that only the presence of P24 in a crude lysate is required to inhibit the antiserum in its abiltiy to precipitate soluble P24 and in its ability to agglutinate preheads.

The requirement for P24 in the particles for agglutination is shown in Fig. 10. A mixture of wild-type phages and 21⁻ preheads was incubated overnight with antiserum, resulting in the aggregation of the phages with the preheads (Fig. 10b). When the same experiment was performed substituting Byp24 (which lacks P24, see Fig. 4) for the wild-type phages, the P24-lacking phages failed to aggregate with the preheads (Fig. 10a) or to aggregate together (Fig. 10c). These experiments confirm that both antibody to P24 and P24 in the particles are required for the observed vertex-to-vertex agglutination of T4 capsids and preheads.

DISCUSSION

Five genes essential for T4 head formation are clustered on the genetic map, numbered 20 through 24. Of these, the functions of three have been demonstrated. P23 is the major

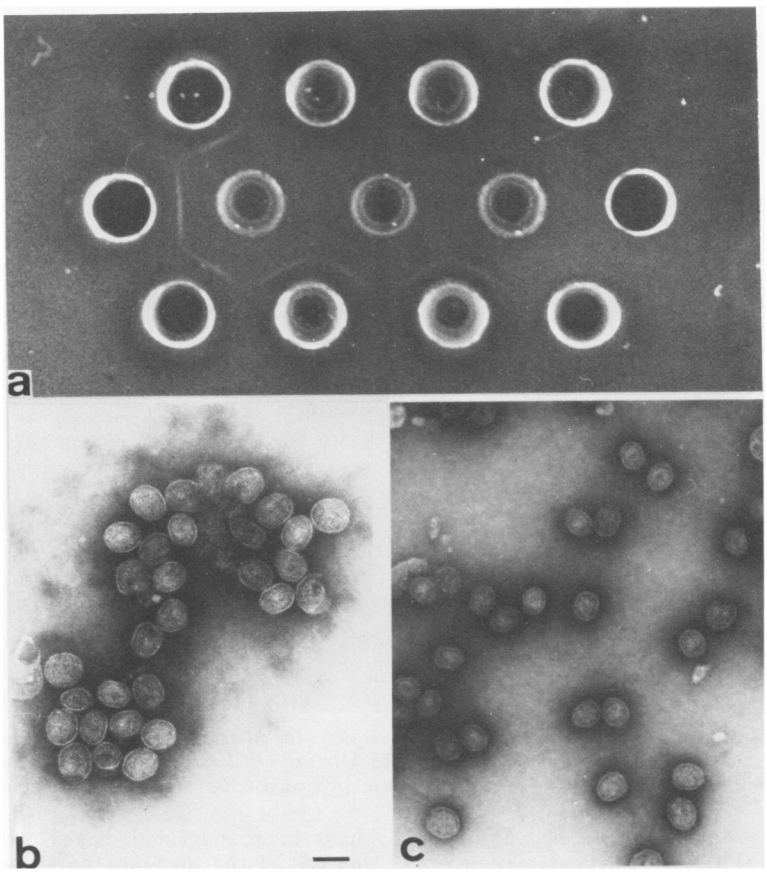

FIG. 9. *Specificity of anti-P24 serum, as judged by Ouchterlony plates and prehead agglutination. (a) Ouchterlony double-diffusion plate with 10 μl of appropriately absorbed serum or antigen-containing lysate in each sample well. Top row: 100 μl of anti-P24 absorbed with (left to right) 10, 25, 50, and 100 μl of a 500-fold-concentrated 23(amH11) defective lysate. Center row (left to right): first well contains the preabsorbed anti-P24 (100 μl of antiserum absorbed with 100 μl of 100-fold-concentrated 24(N65) defective lysate). The remaining wells contain 125-fold-concentrated 23(amH11) lysate. Bottom row: 100 μl of anti-P24 absorbed with (left to right) 50, 100, 200, and 400 μl of 500-fold-concentrated 23(amH11)-24(amN65) lysate. (b) 21(tsN8) preheads incubated 30 min with absorbed serum prepared from 100 μl of anti-P24 plus 400 μl of 500 × 23-24 defective lysate. The preheads still aggregate. (c) 21(tsN8) preheads incubated 30 min with absorbed serum prepared from 150 μl of anti-P24 plus 100 μl of 500-fold-concentrated 23(amH11) defective lysate. The preheads do not agglutinate. Bar = 0.1 μm for both micrographs.*

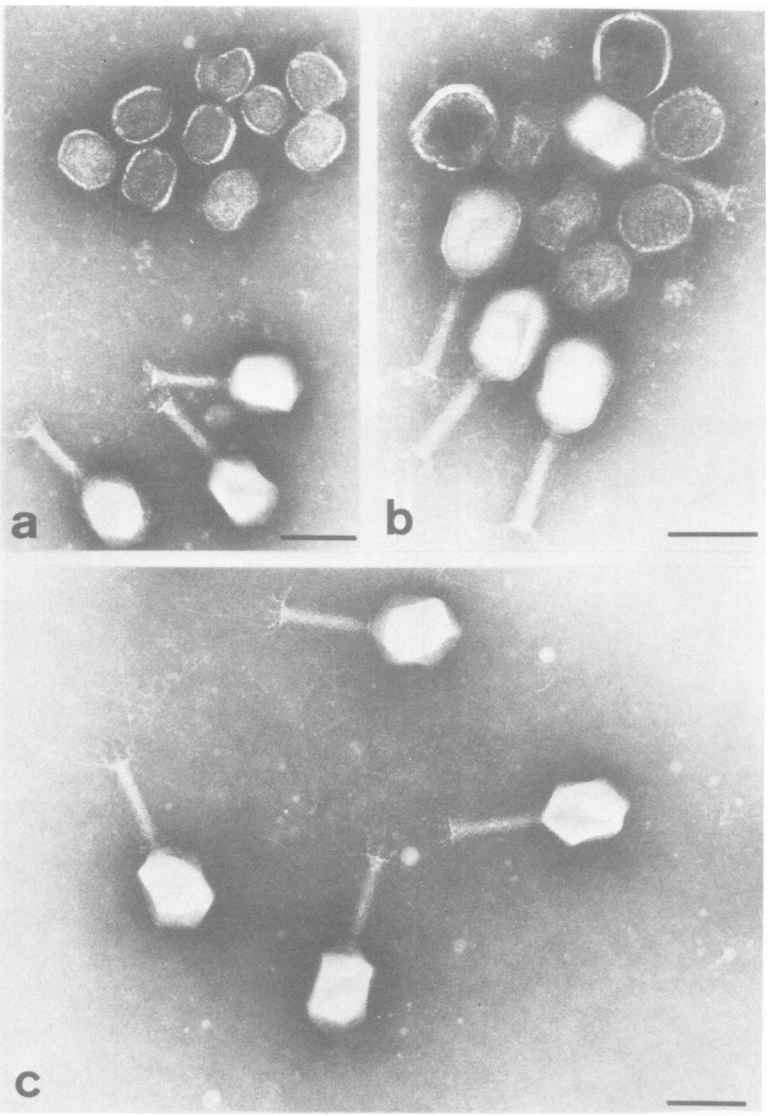

FIG. 10. *Requirement of P24 in phage for agglutination. (a) Mixture of 21(tsN8) preheads and Byp24 mutant phage incubated overnight with anti-P24. (b) Mixture of 21(tsN8) preheads and wild-type phage incubated overnight with anti-P24. (c) Byp24 phages incubated overnight with anti-P24. Bar = 0.1 μm.*

structural protein of the prehead and, in its cleaved form (P23*), of the mature capsid (5, 20). P21 and P22 have transient functions in the prehead. P22 is a scaffolding protein around which the prehead is formed (21, 28), whereas gene 21 codes for a proteinase that cleaves prehead proteins, including itself (29, 30). During head maturation both P21 and P22 are cleaved to small fragments and eliminated from the capsid. The remaining two genes of the cluster, P20 and P24, are constituents of the mature capsid, the latter in its cleaved form, P24* (20). Functions and locations for these two proteins have been suggested, based primarily on the nature of the aberrant particles produced by mutants in the genes coding for them (22). Our studies now provide direct evidence on their location in the prehead and mature capsid and suggest roles for them in head formation and maturation.

Most of the T4 capsid proteins are resistant to removal from the structure by SDS until the temperature is raised to 65 to 70°C, at which point the capsid dissolves. The only exception we have observed is a structure at one end of the capsid, which we presume to be the tail

attachment site, or proximal vertex. This neck usually appears as a swelling, from which short fibers can often be seen protruding (10). This structure is removed from the capsid at 55°C or less in 1% SDS, and its loss is accompanied by the extraction of P20 and a protein we refer to as Px, with a molecular weight of 33,000 based on its mobility on SDS gels. Other minor capsid proteins may be removed as well.

Capsids behave differently when incubated in 7 M urea at alkaline pH. As the pH is raised, gaps appear at the vertexes, and at pH 11.1 the nonproximal vertexes appear to be completely removed. However, the neck structure at the proximal vertex is resistant to this treatment. Many of the capsids extracted at pH 11.1 can be seen to retain a swelling, and sometimes fibers, at the proximal vertex, and these residual capsids retain most of their P20 and Px. Thus, P20 and Px are associated with, or at least protected from, extraction by the neck structure at the base of the phage capsid even when the capsid has been altered by removal of the nonproximal vertexes.

Since mutants in gene 20 make polyhead tubes and no preheads or closed head-related structures, it has been suggested that P20 is associated with the formation of the "hemispherical caps" of phage particles (17, 22). Given the estimate of 10 to 20 molecules of P20 in the capsid (2), a structural role with 1 molecule at each of the 12 vertexes seemed likely. However, our data show that P20 is not at all of the vertexes, since it remains associated with the capsids when the 11 nonproximal vertexes are removed. We suggest that it is located at or around a single vertex position, the normal tail attachment site. Its function in cap formation, then, must not be structural in the sense that it is a part of the vertexes, like the pentons of adenoviruses (34). It seems more likely that it serves to establish a point of fivefold symmetry that nucleates cap formation, starting from the proximal vertex. This nucleation must determine the (u, v = 15, 5) folding that distinguishes normal phages from polyheads (2, 8, 31). The participation of the products of genes 22 and 40 is also suggested by the fact that mutants in these genes are also unable to make structures with the normal (u, v = 15, 5) folding (31, 35).

It is not clear from our results whether P20 is required at the tail attachment site. The presence in wild-type lysates of small numbers of multitailed phages (7, 24) suggests the possibility that it is the absence of P24 from the proximal vertex that allows the tail to attach.

Unlike P20, P24* appears to be localized specifically at the nonproximal vertexes of T4 capsids. This protein is quantitatively removed from capsids by extraction with 7 M urea at pH 11.1, concomitant with the appearance of gaps at these vertex positions. The fact that the proximal vertex position has a different structure suggests that it does not contain P24*. Whenever P24* is extracted with urea, some P23* and *hoc* are also solubilized. It seems reasonable to us to suggest that this represents molecules also coming from the vertex regions, perhaps in contact with the P24*.

The location of P24 and P24*, respectively, at the nonproximal vertexes of preheads and phages is confirmed by the specific binding of antibodies directed against P24 at these positions. By this criterion there seems not to be P24 at the proximal vertex, but the neck structure might mask any P24 present from antibody.

The difference in antibody binding between preheads and capsids requires some comment. The difference may be attributable to the facts that the antiserum was prepared by injection of P24 and that binding sites no longer are present in P24* because its tertiary structure is altered after cleavage. Another possibility is that some antibody binding sites are no longer exposed on the surface of the particle after the prehead has matured to a capsid because they have been covered by parts of neighboring molecules of P23*. Either explanation is compatible with the observed increase in antibody binding to residual capsids from which some P24* and some P23* have been extracted by treatment with 7 M urea at pH 10.9.

What structure can be proposed for the vertexes of the T4 prehead and capsid? We suggest that the 11 nonproximal vertexes of the prehead are each composed of a pentamer of P24, whereas the rest of the surface lattice is composed of hexamers of P23. After cleavage of the prehead shell and core proteins, the expansion of the P23 lattice results in the covering of some of the antibody-binding sites on the P24* at the vertexes. The "eyebrow"-shaped protuberances identified with the vertex positions of bacteriophage T2 seen in freeze-etched preparations (8) are in agreement with a vertex structure in which the circumvertex hexamers partially cover the vertex pentamer.

If P24 is present only in the form of pentamers at the nonproximal vertexes, there should be 55 copies per prehead or capsid. We previously reported capsids to contain 170 molecules of P24* per particle (2). However, recent experiments, using antiserum to identify the P24 band on SDS gels, give a value of 6 molecules of P24* per 100 molecules of P23* in capsids (L. Onorato, manuscript in preparation). This

value agrees with our structure, since current models for the T-even caps d propose 840 (8) or 960 (3) molecules in hexamers (i.e., P23*) per head.

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