Bacteriophage P22 Is Not a Likely Probe for Zones of Adhesion Between the Inner and Outer Membranes of *Salmonella typhimurium*

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Received for publication 21 February 1978

Thin-section electron micrographs of plasmolyzed *Salmonella typhimurium* infected with bacteriophage P22 demonstrated that phage adsorbed to cells over sites of inner- and outer-membrane contact. Efforts were made to isolate such adsorption sites by infection of cells with 35S- and 32P-labeled phage and by separation of the membranes on sucrose gradients. At 37°C, about 75% of the 35S radioactivity could be recovered in a region of intermediate density between the inner and outer membranes. This region (φ band) did not contain 32P. The gradient profile was independent of the multiplicity of infection (between 0.2 and 50) and of the presence or absence of chloramphenicol, dinitrophenol, or cyanide. However, ethylenediaminetetraacetate, when present during the infection step, prevented the formation of φ band. The density of φ band was at least 1.30 g/cm³, as demonstrated by prolonged centrifugation on a D2O-sucrose gradient. φ Band was shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electron microscopy to contain empty phage heads and contaminating cellular debris. In purified preparations, phage heads were the only structures visible by negative staining, and very little cellular phospholipid or protein was associated with the phage proteins (<2% and 30% by weight, respectively, as determined by using [3H]glycerol or [3H]leucine). The residual cellular protein included all of the major outer-membrane proteins rather than any one specific protein. These results are interpreted as indicating that φ band probably does not contain adhesion site material stably associated with phage heads.

One of the outstanding problems of bacterial membrane biogenesis is the mechanism by which phospholipids, lipopolysaccharide (LPS), and proteins are transported to the outer membrane of gram-negative bacteria from their site of synthesis on the inner membrane. Recent studies have shown that phospholipid translocation is reversible (14), whereas that of LPS is not (14, 17, 20). There is no evidence in bacteria for the existence of phospholipid exchange proteins analogous to those in eukaryotes (6, 14). The discovery by Bayer of 200 to 400 areas of contact, or zones of adhesion, between the inner and outer membranes of *Escherichia coli* K-12 (2) offered a potential solution to the problem of translocation. Mührladt subsequently reported that nascent LPS initially appeared in the outer membrane over zones of adhesion before diffusing laterally over the entire membrane (17). This result clearly implicated zones of adhesion as the translocation route for LPS.

Several attempts have been made to isolate zones of adhesion (26) or LPS insertion sites (16) for biochemical analysis. Some authors have speculated that, after sucrose gradient centrifugation of lysed cells, adhesion site material is associated with the outer membrane (10, 16). On the other hand, Tomita et al. (26) assumed that adhesion sites had a density intermediate between that of the inner and outer membrane. Bayer had earlier demonstrated that bacteriophage adsorbed to cells preferentially over the zones of adhesion and had speculated that these zones acted as the conduits for phage DNA injection into cells (3, 4). Tomita et al. (26) accordingly infected *Salmonella anatum* with 35S-labeled bacteriophage 0.25 and separated the membranes on sucrose gradients. They found that if cells were infected at 4°C, the 35S-labeled phage proteins were recovered with the outer membrane. When the temperature of infection was raised to 35°C, however, the phage proteins moved to a region of intermediate density on sucrose gradients, between the inner and outer membranes. The authors postulated that in the

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latter case, $\text{e}^{15}$ phage were attached to zones of adhesion. These results prompted us to investigate the use of phage as affinity labels for zones of adhesion and of sucrose gradients for purifying attachment sites for biochemical analysis. Using $^{35}$S-labeled bacteriophage P22, which infects smooth strains of *Salmonella typhimurium*, we demonstrated that a $^{35}$S-labeled band appears on sucrose gradients, which is analogous to that reported by Tomita et al. (26). Evidence is presented which suggests that this band consists primarily of empty phage heads and does not represent phage attached to adhesion site material.

**MATERIALS AND METHODS**

Bacterial strains and phages. G30, a galE mutant of *S. typhimurium* LT-2, has been previously described (22). This strain produces smooth LPS when grown in the presence of galactose, thus becoming susceptible to infection by bacteriophage P22. Phage P22 was a clear mutant obtained from L. Rothfield.

**Media.** Cells were routinely grown in M9 medium (9) supplemented with 0.05 volume of proteose peptone-beef extract (30), galactose (1 mM), and ribose (0.1%) as the carbon source. Low-sulfur minimal medium was M9 salts medium with the sulfate concentration adjusted to 0.17 mM and supplemented with glucose (0.5%) and galactose (1 mM). Low-phosphate minimal medium was as described by Jones and Osborn (14), except that the phosphate concentration was 0.2 mM and galactose (1 mM) was added. All cultures were grown at 37°C. For $^3$H or $^{14}$C labeling of cells, cultures were grown with radioactive precursors for at least three generations.

**Radiochemicals.** [2-$^3$H]glycerol (7.5 mcCi/μmol), $^3$H-$^32$SO$_4$ (carrier-free; 4.3 Ci/μmol), and $^{32}$PO$_4$ (carrier-free) were from New England Nuclear Corp. L-[U-$^3$H]leucine (0.31 mcCi/μmol), L-[4,5-$^3$H]leucine (61 mcCi/μmol), and [5-$^3$H]uracil (27 mcCi/μmol) were purchased from Schwarz/Mann.

**Preparation of $^{35}$S- and $^{32}$P-labeled phage.** G30 cells were grown to a density of 5 x $10^8$ cells per ml in low-sulfur or low-phosphate minimal medium (80 ml) and mixed with P22 phage at a multiplicity of 5. Either 2.5 mcCi of H$_2$SO$_4$ or 2 mcCi of H$_3$PO$_4$ was added 10 min after infection, and growth was continued for 2 to 3 more h. The cells were lysed with chloroform, and phage were purified from the supernatant by sedimentation (23) and isopycnic centrifugation in a CsCl gradient containing 0.1% Triton X-100 (vol/vol) (28). Purified phage had initial specific activities of about $10^5$ cpm per plaque-forming unit (9S) and $5 \times 10^8$ cpm per plaque-forming unit (3P). The $^{32}$P-labeled phage initially contained about 2 $^{32}$P atoms per plaque-forming unit. Phage titration was by the agar layer technique (1).

**P22 infection of G30 and membrane separation.** Exponentially growing cells (100 to 200 ml, 5 x $10^8$ cells per ml) were spun briefly and routinely suspended in 5 ml of prewarmed growth medium containing chloramphenicol (100 μg/ml). After 5 min, P22 phage were added at a multiplicity of infection of between 0.2 and 50 and allowed to infect cells, usually for 5 min at 37°C. Cells with bound phage were washed twice at 4°C in N-2-hydroxyethyl piperezine-N'-2-ethanesulfonic acid (HEPES) buffer (10 mM HEPES, pH 7.4); over 99% of the phage radioactivity remained adsorbed even when the initial infection was at 4°C or in tri(hydroxymethyl)aminomethane (Tris)-hydrochloride-ethylenediaminetetraacetate (EDTA) buffer. Cells were suspended in 1 to 2 ml of HEPES buffer containing 25% sucrose, treated with deoxyribonuclease and ribonuclease (5 μg each), and lysed by passage through a French pressure cell at 15,000 lb/in$^2$ as described by Jones and Osborn (14). For some of the experiments with $^{32}$P-labeled phage P22, deoxyribo-

**RESULTS**

Adsorption of bacteriophage P22 to *S. typhimurium*. Thin sections of P22-infected G30 cells (grown in the presence of galactose and therefore phenotypically smooth) showed that phage heads were attached to cells directly over zones of adhesion, when these were clearly resolved (Fig. 1), in the same manner as has been reported for other phages (3).
G30 cells were infected with $^{35}$S-labeled phage P22, and the membranes were separated on sucrose gradients as described above. Figure 2a shows that after 5 min of infection at 37°C, over 70% of the phage radioactivity was recovered at a density ($\rho = 1.22 - 1.23 \text{ g/cm}^3$) intermediate between that of the inner and outer membranes. We will refer to this intermediate $^{35}$S-labeled band as \( \phi \) band. The remaining 20 to 30% of $^{35}$S was apparently associated with the outer membrane ($\rho = 1.275 \text{ g/cm}^3$). Purified phage P22 sedimented in parallel gradients to a density of 1.29 g/cm$^3$ or greater (data not shown). The sucrose gradient profile of Fig. 2a was independent of the multiplicity of infection (between 0.2 and 50) and the presence or absence of chloramphenicol (100 \( \mu \)g/ml), 2,4-dinitrophenol (10 mM), or sodium cyanide (10 mM). Similar profiles were observed after 10-min infections. \( \phi \) Band always accounted for 60 to 75% of the phage radioactivity applied to the gradient.

When cells were infected with $^{32}$P-labeled phage, no $^{32}$P was recovered in \( \phi \) band, indicating that this band did not contain phage P22 DNA. In this experiment deoxyribonuclease was omitted from the French pressing procedure. Similar results were obtained when EDTA was present during the lysis procedure and in the sucrose gradient, to inhibit endogenous nucleases.

**Conditions preventing the formation of \( \phi \) band.** When cells were infected with P22 in the presence of Tris-EDTA buffer for 5 min at 37°C and the membranes separated, little \( \phi \) band was formed (<15% of the $^{35}$S counts, Fig. 2b). Most of the phage radioactivity recovered was either associated with the outer membrane or in denser fractions. In contrast, cells infected at 37°C in the presence of Tris buffer alone yielded a sucrose gradient profile closely resembling that of Fig. 2a.

The formation of \( \phi \) band was prevented under all other infection conditions tested. These were (i) infection of G30 cells with P22 for 15 s at 37°C followed by 5 min at 4°C and (ii) incubation of a French press lysate of cells with P22 for 10 min at 37°C. It made little difference whether or not, in the latter case, the mixture was passed through the French press for a second time before sucrose gradient centrifugation. In all cases phage radioactivity was recovered in association with the outer membrane rather than in the position characteristic of free phage.

To determine whether the formation of \( \phi \) band was correlated with irreversible phage adsorption, we incubated P22 with cells and measured the extent of irreversible binding after 10 min (Table 1). Control experiments indicated that in each case over 99% of the phage cosedimented...
with cells under these conditions. Although the extent of irreversible binding was not directly correlated with the formation of \( \Phi \) band, the data suggested that irreversible binding was necessary (but not sufficient) for \( \Phi \) band formation.

**Characterization of \( \Phi \) band.** (i) **Cellular phospholipid content.** To test whether \( \Phi \) band was at an equilibrium density in the sucrose gradient and to quantitate the amount of cellular phospholipid associated with it, G30 cells were labeled for three generations with 5 mCi of [2-\( ^3 \)H]glycerol and infected with 35S-labeled P22 at a multiplicity of 25. \( \Phi \) Band was collected at a density of 1.22 g/cm\(^3\) after separation of the membranes on a sucrose gradient (16 h, 40,000 rpm), diluted, and relayered onto a 20 to 50% sucrose gradient prepared with D\(_2\)O. After centrifugation for 112 h at 40,000 rpm, \( \Phi \) band was recovered at a density of 1.30 g/cm\(^3\), indicating that it had been far from equilibrium in the water-sucrose gradient. The band was pooled and rerun on a water-sucrose gradient under normal conditions (16 h, 40,000 rpm). Its apparent buoyant density was still 1.22 g/cm\(^3\) on this gradient. \( \Phi \) Band was run on three further consecutive gradients (16 h, 40,000 rpm) to separate it as much as possible from an overlapping peak of phospholipid. After the \( \Phi \) band was run on the

**FIG. 2. Formation of \( \Phi \) band after sucrose gradient centrifugation of lysates prepared from P22-infected cells.** (a) Cells infected at a multiplicity of 0.2 in growth medium containing 100 \( \mu \)g of chloramphenicol per ml. (b) Cells infected at a multiplicity of 5 in 100 mM Tris-hydrochloride (pH 8.0), containing 5 mM EDTA. Both infections were for 5 min at 37°C. Cells were labeled with [2-\( ^3 \)H]glycerol (\( \bullet \)), and P22 was labeled with 35S (\( \circ \)).

<table>
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<tr>
<th>Infection conditions*</th>
<th>Reversibly bound phage (%)</th>
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<tbody>
<tr>
<td>M9, 37°C</td>
<td>0.3</td>
</tr>
<tr>
<td>Tris, 37°C</td>
<td>5</td>
</tr>
<tr>
<td>M9, 4°C</td>
<td>11</td>
</tr>
<tr>
<td>Tris-EDTA, 37°C</td>
<td>16</td>
</tr>
<tr>
<td>Tris, 37°C, with cells lysed by French pressure cell</td>
<td>77</td>
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</table>

*Exponentially growing cells were concentrated 20-fold and incubated with P22 for 10 min at a multiplicity of 1.0 under the conditions indicated. M9 was the supplemented minimal medium used for growing cells. Tris and Tris-EDTA were as described for Fig. 2.

*Portions of the infected cells were mixed with a drop of CHCl\(_3\) and titrated for residual viable phage (15).*
final gradient (Fig. 3), this overlapping phospholipid peak was still detectable. From the $^{3}$H/$^{35}$S ratio in the peak fraction of $\phi$ band (fraction 8), phospholipid represented at most 2% of the weight of the phage proteins, assuming that the cellular phospholipid was uniformly labeled with $[^3]$Hglycerol and that the specific activity of the phage proteins in $\phi$ band was the same as that of the intact phage. The latter assumption is reasonable since $\phi$ band consists almost entirely of the major coat protein of P22, which constitutes over 75%, by weight, of the P22 proteins (8; see below).

(ii) Phage protein content and electron microscopy. Cells were infected with $^{35}$S-labeled phage P22 at a multiplicity of 50 for 5 min at 37°C, and the membranes were separated on a sucrose gradient. Portions from the peak fractions of $\phi$ band and the outer-membrane-associated band (OM band) were run on an SDSPolyacrylamide slab gel (Fig. 4). $\phi$ Band contained only two major phage proteins: the two structural proteins of the P22 head (P1 and P5 according to the nomenclature of Botstein et al. [8]). The OM band contained P9 and P20 in addition to P1 and P5; P16 was missing from both bands. Two other low-molecular-weight P22 proteins (P26 and PX) showed up only weakly in autoradiograms of pure $^{35}$S-labeled phage; we could not be certain whether or not these proteins were present in either the $\phi$ band or OM band.

Electron micrographs prepared from $\phi$ band and OM band are shown in Fig. 5a and b. Phage heads were observed in both preparations. The phage in OM band were, as expected, attached to outer-membrane fragments. Some similar structures were also present in $\phi$ band; however, most of the phage heads were not attached to the large amount of contaminating membranous material in this fraction.

![Fig. 3. Association of cellular phospholipid with $\phi$ band purified by multiple density-gradient centrifugations. The apparent buoyant density of $\phi$ band is 1.22 g/cm$^3$. Symbols: O, $^3$H-labeled phospholipids; O, $^{35}$S-labeled phage proteins.](image)

**Fig. 4. Phage protein content of $\phi$ band and OM band. Autoradiograms of the $^{35}$S-labeled protein were prepared after SDS-polyacrylamide gel electrophoresis. (1) Purified phage P22; (2) $\phi$ band; (3) OM band. Molecular weights of the phage P22 proteins are from Botstein et al. (8).**

**Purification of $\phi$ band.** To test whether the phage heads in $\phi$ band were attached to specific cellular proteins, we developed a rapid method for purifying $\phi$ band. Preliminary experiments showed that fractions in the $\phi$ band region of sucrose gradients were contaminated by a peak rich in cellular protein and RNA (labeled with $[^3]$H]leucine and $[^5]$C]uracil, respectively), as expected if ribosomes were present (Fig. 6a). This peak was substantially removed when the French press lysate was pretreated with 5 mM EDTA and subjected to a preliminary centrifugation step, as described above (Fig. 6b). This treatment was therefore adopted as the first step in purifying $\phi$ band in order to avoid contamination of $\phi$ band with ribosomes, which are similar in size to phage heads. It should be emphasized that the membranes had to be sedimented onto a sucrose cushion during the preliminary centrifugation step; attempts to sediment the membranes as a pellet led to the formation of a large M band (13) in exactly the
same position as φ band (data not shown). Figure 7 shows an SDS gel electropherogram of proteins from fractions pooled and concentrated from the ρ = 1.19 – 1.21 g/cm³ region of the gradients shown in Fig. 6. EDTA clearly eliminates all of the major low-molecular-weight proteins found in this region.

φ Band was isolated, by using the procedure described above, from cells that had been heavily labeled with [³H]leucine and infected with ⁴⁵S-P22 at a multiplicity of 50. Half of a culture grown in the presence of 2 mCi of [³H]leucine was treated in this way; the other half acted as a control. The fractions from a sucrose gradient of the uninfected control cells that were equivalent to the φ band-containing fractions of the infected cells were pooled. Both pooled fractions were purified by passage through a series of polycarbonate filters with decreasing pore sizes. Figure 8 summarizes the recovery of cellular and phage protein at each step of the purification procedure. The overall recovery of ⁴⁵S was about 50%; 75 to 100% of the ⁴⁵S counts were recovered in each step. Of the recovered counts, about 50% were collected from the 0.05-μm-pore filter after extensive washing and 25% were collected in the filtrate. Only the washed fraction contained more ³H than the equivalent fraction in the control experiment.

SDS gels of these fractions are shown in Fig. 9. Only in the material washed from the 0.05-μm filter did the ⁴⁵S-containing sample have more

**FIG. 5.** Negative staining of fractions from (a) φ band, (b) OM band, and (c) purified φ band. Empty phage heads are densely stained (arrows). Bar, 0.1 μm.
Fig. 6. Sucrose gradient centrifugation of cell lysates labeled in protein and RNA. Lysates were subjected to preliminary centrifugation (3 h, 50,000 rpm) onto a 60% sucrose cushion to remove soluble proteins before layering the membrane-containing fractions on the standard sucrose gradient. (a) EDTA was omitted from the preliminary centrifugation. (b) Lysates were treated with 5 mM EDTA for 3 h and subjected to preliminary centrifugation in the presence of 5 mM EDTA, as described in the text. Symbols: ○, [14C]leucine; ○, [3H]uracil.

3H-labeled protein bands than did the control sample. This fraction of the control sample lacked any discernible protein bands (Fig. 9, lane 6), whereas the equivalent fraction of the phage protein-containing sample contained a detectable amount of the major outer-membrane proteins (Fig. 9, lane 5). From the ratio of 3H/35S in this fraction it could be calculated that cellular proteins represented, at most, 30% of the weight of the phage proteins, assuming that the cellular proteins were labeled with [3H]leucine to the same specific activity as the total cell protein. In electron micrographs of this purified φ band material, the majority of phage heads were not attached to any visible structure (Fig. 5c). However, a small fraction (<10%) seemed to be associated with material which could have been residual outer membrane (Fig. 5c, top left corner).

DISCUSSION

The existence of areas of adhesion between the inner and outer membrane and their role as sites of irreversible phage adsorption and of LPS translocation to the outer membrane have been demonstrated only by electron microscopy (2, 3, 17). The isolation and biochemical analysis of material originating from zones of adhesion have not yet been reported. Kulpa and Leive (16) have isolated, from sucrose gradients, outer-membrane fragments enriched for newly synthesized LPS and (by implication) for presumptive adhesion site material. On the other hand, Tomita et al. (26) assumed that adhesion site material should have a density intermediate between that of the inner and outer membranes. These authors infected S. anatum with bacteriophage ε15 and, after separating the membranes on sucrose gradients, recovered all the phage protein in a band of intermediate density. They concluded that the phage was probably bound to adhesion site material.

By infecting S. typhimurium with bacteriophage P22, we were able to generate similar, though not identical, results: 60 to 75% of the radioactivity from 35S-labeled phage was re-
Tris-EDTA buffer at 37°C, in growth medium at 4°C, or to lysed cells, \( \phi \) band did not form. Similar conditions (i.e., phage adsorption at 4 or 15°C) lead to completely reversible binding in some other systems (19, 25); furthermore, Kagenasaki and Tomita (15) isolated a mutant of \( S. anatum \) to which \( \epsilon^{15} \) bound reversibly at 37°C, under conditions in which no \( \phi \) band was formed (26). These results suggest that irreversible binding is necessary for the formation of \( \phi \) band in both the \( S. anatum-e^{15} \) and \( S. typhimurium-P22 \) systems. Since \( \phi \) band did not contain P22 DNA in the present experiments and since phage adsorption is by definition irreversible when bound phage interact with zones of adhesion and inject their DNA, the results are also consistent with the hypothesis proposed by Tomita et al. (26) and with that outlined above.

According to the hypothesis proposed by Tomita et al., the density at which \( ^{35}S \)-labeled phage proteins are recovered after sucrose gradient centrifugation is attributable to the fact that empty phage heads are attached to a relatively large mass of adhesion site material having that buoyant density. However, we have shown, using \( D_2O \)-sucrose gradients, that \( \phi \) band is not at an equilibrium density in the standard water-sucrose gradient and that the phage proteins in \( \phi \) band are stably associated with (at most) 2%, by weight, phospholipid. These results were confirmed by electron microscopy of \( \phi \) band material, both as isolated from the initial sucrose gradient and after purification using the polycarbonate filter method. Purified \( \phi \) band appeared to consist of empty collapsed phage heads; almost no attached structures were visible. However, fluorography of \( \phi \) band proteins after SDS-polyacrylamide gel electrophoresis showed that, in the most highly purified fraction, small amounts of \( ^3H \)-labeled outer-membrane proteins accompanied the \( ^{35}S \)-labeled phage head proteins. If this association were specific, one would hope to be able to cross-link the proteins chemically. We performed cross-linking experiments on cell lysates and purified \( \phi \) band material by using the diimidodester dimethylsuberimidate at high concentrations, but were unable to detect any cross-linked proteins on gels (data not shown). We therefore have no evidence for any specific association between outer-membrane proteins and phage heads.

Since EDTA treatment was included in the purification procedure, it is possible that we missed seeing proteins whose attachment to phage heads might depend upon the presence of divalent cations. However, any such EDTA-sensitive attachment cannot involve aggregates visible by negative staining, since the \( \phi \) band ma-
Recovery of $\text{H}$-Cellular proteins and $\text{S}$-phage proteins

<table>
<thead>
<tr>
<th>Purification Step</th>
<th>Recovery of $\text{H}$-Cellular proteins</th>
<th>Recovery of $\text{S}$-phage proteins</th>
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<tr>
<td></td>
<td>(cpm x $10^3$)</td>
<td>(cpm x $10^3$)</td>
</tr>
<tr>
<td>1. $\phi$ band</td>
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<td>140</td>
</tr>
<tr>
<td>2. Filtration (0.45 $\mu$m pore filter)</td>
<td>405 (F) 1280 (R)</td>
<td>3500 1720 (R)</td>
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<tr>
<td>3. Dialysis</td>
<td>300</td>
<td>220</td>
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<tr>
<td>4. Filtration (0.08 $\mu$m pore filter)</td>
<td>200 (F) 34 (R)</td>
<td>100 (F) 42 (R)</td>
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<tr>
<td>5. Filtration (0.05 $\mu$m pore filter)</td>
<td>136 (F) 27 (R)</td>
<td>22 (F) 49 (R)</td>
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<tr>
<td>6. Wash (of 0.05 $\mu$m pore filter)</td>
<td>3 (W) 24 (R)</td>
<td>24 (W) 25 (R)</td>
</tr>
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</table>

**Fig. 8.** Filter purification of $\phi$ band. Fractions from sucrose density gradients were filtered through 0.45-$\mu$m membrane filters (Millipore Corp.; type HAWF), dialyzed against water to remove sucrose, and squeezed in 2-ml portions through 0.08-$\mu$m Bio-Rad Uni-Pore polycarbonate membrane filters held in Swinnex filter holders. Filtrates were passed through 0.05-$\mu$m polycarbonate membrane filters. These filters were washed three times with 5 ml of water. $+\text{P22}$, $\phi$ Band isolated from P22 infected cells; $-\text{P22}$, control experiment in which equivalent fractions from uninfected cells were subjected to the same purification procedure. F, Filtrate; R, retained on filter; W, washed from filter.

which might be expected to be gentler than French pressing, only 40% of the phase counts were recovered in $\phi$ band (data not shown), in support of this model.

Jazwinski et al. (12) have reported that when *E. coli* K-12 is infected with $\phi$X174 and the membranes are separated on sucrose gradients after French press lysis, both phage particles and phage DNA band in the outer-membrane region. Closer analysis of their data (their Fig. 6) suggests that phage DNA is recovered in a region of intermediate density. We have repeated these experiments by using $\phi$X174 infection of *S. typhimurium*. Our results confirm those of Jazwinski et al. (12) in that both phage DNA and phage protein were recovered between the inner and outer membranes, although some radioactivity ($<20\%$) from $32P$-labeled phage was also recovered in the inner- and outer-membrane fractions (data not shown). However, in control experiments intact $\phi$X174 also sedimented between the inner and outer membranes. Phage titrations of the intermediate density fractions demonstrated that the intermediate peak of $\phi$X174 protein and DNA was composed of two adjacent peaks of viable and eclipsed phage (data not shown). The suggestion that $\phi$X174 DNA was bound to adhesion site material (12) therefore seems unfounded.
In summary, we feel that phage attachment to adhesion sites does not provide a practical method for isolating adhesion site material. Our results do not disprove that the residual cellular material associated with ϕ band originates from the zones of adhesion, but we feel it is unlikely to be specifically associated adhesion site material, especially since it is clear from electron micrographs that only the phage base plate is in direct contact with the cell; the phage heads are about 10 nm away from the outer membrane (Fig. 1). A major assumption in this work, and in those of other authors, has been that adhesion sites are stable, relatively homogeneous structures, with a composition sufficiently different from that of the surrounding membrane to permit their recognition. There is still no evidence to support this assumption.

ACKNOWLEDGMENT

We are grateful to Kathryn Book for carrying out the electron microscopy.

LITERATURE CITED


Fig. 9. Fluorograms of purified ϕ band material after SDS-polyacrylamide gel electrophoresis. Protein samples purified as described in Fig. 8 were concentrated by lyophilization before electrophoresis. (1 and 2) Partially purified material (dialysate from step 3 described in Fig. 8); (3 and 4) filtrate from the 0.05-μm-pore filter (following step 5 described in Fig. 8); (5 and 6) pooled washings from the 0.05-μm-pore filter (following step 6 described in Fig. 8). (1, 3, and 5) Samples from P22-infected cells; (2, 4, and 6) samples from uninfected (control) cells. Samples loaded onto gels represent 10% of the total material at each stage except for slot 3 (45% of the material) and slot 6 (30% of the material). The counts per minute for 3H and 35S loaded in each slot are indicated below the gel.