The Virion Proteins and Ultrastructure of Staphylococcus aureus Bacteriophages

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SUMMARY

The number and size of the major virion polypeptides have been determined by SDS-PAGE for the 22 Staphylococcus aureus phages of the International Typing Set, plus phages 11 and 80z. Virion ultrastructure was examined by electron microscopy after negative staining with ammonium molybdate. In addition, serogroup B phages were disrupted and fractionated into head, tail-tube and baseplate components and major polypeptides assigned to these substructures. The number and size of the polypeptides correlated closely with the division of aureophages into four serogroups (A, B, F, L), although serogroup L was represented in the set by only a single phage (187). Apart from serogroup B, however, the polypeptide patterns did not reflect differences between lytic groups. Within serogroup B, polypeptide analysis yielded characteristic patterns for lysogroups I, II and III. Ultrastructural analyses confirm the data provided by polypeptide analysis. Thus, phages from the four serogroups can be identified on the basis of tail-tube length alone, although the differences between phage 187 and members of lysogroups I and III in serogroup B were less than 20 nm, or approximately 12% of the total length. Serogroup A virions differed from those of the other serogroups in that all members of the typing set in this group had elongate, rather than isometric, heads.

INTRODUCTION

Bacteriophages of Staphylococcus aureus are classified according to serological and lytic reactions. These two parameters reflect, respectively, differences in the external structure of the virions, and differences in the host range of the phages. The latter is likely to be determined by recognition and attachment structures on the surface of virion and host cell, by restriction/modification systems in the host cell, by phage genetic sequences that recognize enzymes or other factors involved in early steps in phage multiplication, or by other phage or host characteristics.

Some 60 to 70 phages of S. aureus have so far been identified (including the International Typing Set), largely by serological and host range characteristics. These phages are probably representative of a larger number of aureophages. Because aureophages are important elements of gene transfer (transduction, phage-mediated conjugation) and gene expression (lysogenic conversion) in populations of S. aureus, it is likely that they are involved in determining virulence and resistance in clinically significant populations of the bacterium. A clearer knowledge of the taxonomic and phylogenetic relationships among the aureophages will lead to a better understanding of genetic exchange and interaction between phages, between phages and host cells, and between staphylococci.

Previous comparative studies of aureophages have demonstrated varying degrees of DNA homology among selected members of the International Typing Set (Pariza & Iandolo, 1974; Stewart et al., 1985). Homology measurements on total genomes did not correlate highly with serological or lytic relationships, though the numbers of different phages compared were small.

In this study, we report analyses by SDS-PAGE of the virion proteins of the 22 phages in the International Typing Set, plus those of phages 11 and 80z, together with electron microscopic
observations of the structure of the virions. We show that the number and molecular sizes of the virion polypeptides correlate well with serological groupings of these phages, rather than with lytic groupings. We also report, for serogroup B phages, the location of the major polypeptides in the head and tail structures of the virions.

**METHODS**

**Phages and bacterial strains.** Phage 55, its propagating strain (Ps55), ISP 300 (an 8325 lysogen carrying phage 80a) and ISP 8 were obtained from Dr P. Pattee (Iowa State University, Ames, Iowa, U.S.A.). Phage 80a was induced from ISP 300 by irradiation in normal saline to 99% inactivation, followed by phage propagation in trypticase soy broth (TSB; BBL, Cockeysville, Md., U.S.A.) at 37°C.

The phages 80, 83A and 85 and their propagating strains were obtained from Ms A. Vickery (Royal Prince Alfred Hospital, Sydney, Australia; an authorized international distribution centre for *S. aureus* typing phages). Phage 11 was obtained from Dr B. Egan (University of Adelaide, Adelaide, Australia) as a lysogen in strain 8325-4 and was induced by mitomycin C (2μg/ml) to an exponential phase culture.

The other 18 phages and their propagating strains were obtained from the American Type Culture Collection as freeze-dried preparations.

**Propagation of phage.** Overnight cultures of the propagating strains, grown in TSB at 37°C, were diluted 100-fold into fresh broth and shaken at 37°C until the optical density at 550 nm was approximately 0-1 (106 cells/ml). Phage was then added (10 to 107 p.f.u./ml) plus CaCl2 to 4 mM, and the culture incubated at 37°C without shaking for 10 min, to permit phage adsorption, then with shaking until lysis had occurred (2 to 6 h).

**Purification of phage virions.** After lysis, virions were precipitated with polyethylene glycol and pelleted as described by Yamamoto et al. (1970) except that the NaCl concentration was increased to 1-0 M to increase the precipitation efficiency. The pelleted virions were resuspended in buffered CsCl (67 g CsCl in 82 ml 10 mM-NaCl, 50 mm-Tris–HCl pH 7-5, 10 mM-CaCl2), and centrifuged at 38000 r.p.m. in a Beckman SW50.1 rotor for 16 to 20 h at 4°C. Each 5 ml ultracentrifuge tube was loaded with phage from 300 ml of lysate.

The banded phage particles were re-banded in CsCl, as above, then diluted 1 in 5 with 10 mM-NaCl, 50 mm-Tris–HCl pH 7-5, 10 mM-CaCl2 and pelleted by centrifugation in a Beckman SW50.1 rotor at 40000 r.p.m. for 90min. Pellets were resuspended in 0-1% SDS and stored at −20°C until required for electrophoresis. Phage preparations used for separation of heads, tails and baseplates were not pelleted from the final CsCl banding, but were stored in CsCl at 4°C.

**Phage assay.** Phage were assayed by spotting dilutions in TSB plus 4 mM-CaCl2 onto lawns of the appropriate propagating strain in trypticase soy soft agar containing 4 mM-CaCl2. Plates were incubated at 37°C. Strain ISP 8 was used for assay of phages 11 and 80a.

**Polyacrylamide gel electrophoresis.** Electrophoresis was carried out as described by Laemmli (1970). Virion suspensions in SDS–mercaptoethanol were heated to 100°C for 5 min and 3 to 10 μg protein was applied to 0-8 mm-thick gels (10 to 15% acrylamide in the resolving gel). Gels were run at room temperature at 130 V through the stacking gel, at 250 V through the resolving gel, then stained with Coomassie Brilliant Blue R to locate polypeptides. Molecular weights were estimated by reference to standard proteins prepared in the same way and run on the same gels.

Two-dimensional electrophoresis was carried out essentially by the method of O'Farrell (1975) except that pH 3-5 to 10 ampholyte (LKB) was used alone, the anode/cathode geometry was reversed, and the sample was loaded at the anode (acidic) end of the gel. Tubes 85 × 3 mm (internal diam.) were used, and gels from two of these were run in parallel on a single second dimension slab gel (160 × 170 × 0-8 mm; 12% acrylamide).

**Densitometry.** Densitometer traces of the wet gels were obtained using a Transidyne RFT 2955 scanning densitometer at a wavelength of 600 nm.

**Fractionation and purification of phage subfractions.** Phage particles in CsCl were disrupted by adding 0-1 vol. 0-2 M-EDTA, 2 mM-Tris–HCl pH8-5, then an equal volume of formamide and incubating at 37°C for 60 min. After incubation the suspensions contained a mixture of empty heads, tails and baseplates; no intact phage particles were present (except with phages 11 and 80a, as discussed below). Five vol. 10 mM-NaCl, 50 mM-Tris–HCl pH 7-5, 10 mM-CaCl2 was then added and mixed, and 150 μl of the preparation was centrifuged at 85000 g for 15 min in a Beckman Airfuge. The supernatant contained heads and baseplates, and the pellet contained mainly tails which had formed long filaments as described under Results. Baseplates were resolved from heads by centrifuging the incubated mixture at 85000 g for 20 min in the Airfuge. Under these conditions, both heads and tails pelleted, and baseplates remained in the supernatant.

**Electron microscopy.** Formvar-coated grids were placed on a drop of the phage in CsCl, left 60 s, then lifted and dried with filter paper. The grid was then placed on a drop of 2% ammonium molybdate for 60 s, lifted, dried with filter paper, then dried in air.

Grids were examined using an Hitachi 500 transmission electron microscope. Preparations were examined at magnifications of 36000 to 55000. The microscope was calibrated using catalase crystals (periodicity of 8-75 nm and 6-85 nm) and with grid or line grating replicas.
Virion proteins and structure of aureophages

RESULTS

Virion polypeptides resolved by SDS-PAGE.

Fig. 1 illustrates the resolution and relative intensities of staining of virion polypeptides for phages from serological groups F and L. Since it was not possible, on a single gel, to separate polypeptides for all 24 phages examined in this study, combined data from a series of gels are summarized schematically in Fig. 2.

A number of conclusions may be drawn from these data. All phages contain two to four major polypeptides with molecular weights of 20K to 40K. Minor proteins (three to five species) generally have higher molecular weights of 40K to 80K.

Serogroups A and F contain phages with polypeptides characteristic for these two groups and, with minor variations within the serogroups, were generally similar regardless of lytic group. Serogroup B, by contrast, is more diverse and within this group lytic groups were characterized by different polypeptide species. Serogroup L contains only a single phage (187) which was distinct from all the other phages examined.

Phages in the same lytic group, but differing in serological reaction, did not have similar polypeptide compositions. Thus, phages 6, 53 and 77, all from lysogroup III but from serogroups A, B and F respectively, have no polypeptides in common. Lytic characteristics thus may either reflect the properties of minor virion proteins which are not identified in these experiments or may be the outcome of point mutations which result in amino acid changes in major proteins which are not detectable by SDS-PAGE.
Fig. 2. Schematic summary of molecular sizes of virion polypeptides for typing phages, phage 11, and phage 80a. Letters and roman numerals at the top indicate serogroup and lysogroup, respectively. Numbers at the bottom indicate individual phages. The relative thicknesses of the bands indicate the degree of Coomassie Brilliant Blue staining of bands on the original gels.

Phages 11 and 80a, not part of the International Typing Set, are in serogroup B (Novick, 1963, 1967). They have been included in lysogroup III because of similarities in their polypeptide composition to other members of this group. Phages 52A and 80a contained an additional low mol. wt. polypeptide (22K) which was not seen in other serogroup B phages.

Two-dimensional electrophoresis of virion proteins
Isoelectric focusing in urea–ampholyte polyacrylamide gels, followed by electrophoresis in SDS–polyacrylamide gels, gave two-dimensional distributions of polypeptides as illustrated in Fig. 3 for phages representative of the four serogroups. While the positions of individual polypeptide species varied between phages, the polypeptides in each case showed a similar overall pattern: most of the polypeptides were acidic, with isoelectric points in the range pH 4 to 6. A number of minor polypeptide species had isoelectric points of 9 or greater. In some cases, polypeptides seen on single-dimension SDS–polyacrylamide gels were resolved into two species in the second (isoelectric focusing) dimension. Whether this represents real differences (and thus an increase in the number of proteins which constitute each virion), or is due to charge heterogeneity for single protein species, cannot be determined at this point.

Electron microscopy of phages
Fig. 4 illustrates virion structures characteristic of each serological and lytic group. Heads were found to be either spherical or icosahedral in different preparations; the apparently spherical forms probably were originally icosahedral but became rounded during preparation or staining of the phage. Phage heads were seen sometimes as ghosts (presumably lacking DNA) or intact (Fig. 4, serogroup A) as was the case with other phages examined by these methods.
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Fig. 3. Two-dimensional electrophoresis of representative phage virions. The first (isoelectric focusing) dimension was horizontal, with the anode on the left and the cathode on the right. The second (sizing) dimension was vertical, with migration from top to bottom. Markers across the gels, left to right, indicate the isoelectric migration of Aspergillus niger amylglucosidase (native protein pI 3.6), equine myoglobin major band (pI 7.4) and yeast cytochrome c (pI 10.2). The vertical axis markers indicate polypeptide molecular weights of 20000, 40000 and 60000. The phages shown are representative of the four serogroups examined.

According to the shape of the virion heads, the aureophages examined can be divided into two morphological groups. Serogroup A phages have an elongated head whereas serogroups B, F and L have an isometric head. On the basis of tail-tube length, phages in serogroup B, F and L can be further subdivided (Table 1). Phages in serogroups B and L had short tails (130 to 170 nm) and those in serogroup F had medium length tails (210 nm). Phage in serogroup B can be further subdivided on the basis of tail length as shown in Table 1.

Although the resolution of baseplate structure was not as clear as that for heads and tail-tubes, serogroup A phages appeared to have a baseplate ending in six pins or spokes (about 10 nm) and those of serogroups B and L had baseplates ending in six pins of slightly greater length (about 15 nm). Tails from serogroup F phages appeared to end in a small knob.

Disruption of virions and separation of heads, tails and baseplates

A number of methods were used in an attempt to disrupt virions into their structural elements. The most effective means of disaggregating head, tail-tube and baseplate was by incubation in 0.02 M-EDTA, 0.2 M-Tris–HCl pH 8.5, 50% formamide at 37 °C for 60 min. Although this
**Virion proteins and structure of aureophages**

Table 1. Head and tail sizes of aureophage virions*

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Lysogroup</th>
<th>Head diameter (nm)</th>
<th>Tail-tube length (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>II</td>
<td>77 ± 2.6</td>
<td>270 ± 9.6</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>x 42 ± 1.6</td>
<td>(elongate)</td>
</tr>
<tr>
<td></td>
<td>(3A, 3C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>III</td>
<td>54 ± 0.8</td>
<td>135 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>(6, 42E, 47, 54, 75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Misc.†</td>
<td>54 ± 0.6</td>
<td>169 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>(81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>I</td>
<td>54 ± 0.9</td>
<td>128 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(29, 52, 52A, 79, 80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>54 ± 0.6</td>
<td>169 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>(11, 53, 80a, 83A, 85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>III</td>
<td>54 ± 0.8</td>
<td>212 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(77, 84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(42D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>Misc.</td>
<td>54 ± 0.7</td>
<td>150 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>(187)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* A minimum of 20 measurements of both heads and tails was made for each phage virion. Tail-tube lengths were measured rather than whole tail (tube plus baseplate) since distortion of the latter caused considerable variation in the measurements. Phage identification numbers are given in parentheses in lysogroup column. Values given are mean and standard deviation.
† Miscellaneous.

Treatment was successfully employed with virions from the four serogroups, as determined by counting virions and substructures in random fields in the electron microscope, two serogroup B phages (11 and 80a) were not susceptible; treatment resulted only in loss of DNA from the head and dissociation of the baseplate from the virion.

Methods such as vortexing (5 min) and sonication (2.5 min) failed to disaggregate the particles. Passage through a French pressure cell at 1400 kg/cm² dissociated 90% of the particles into tail and head when this treatment was applied to phage from filtered lysates (i.e. immediately after lysis of cells); CsCl-purified phage did not dissociate under these conditions.

After virion disruption, tails were polymerized and separated from heads and baseplates as described in Methods. Polymerized tail-tubes are illustrated in Fig. 5, and the polypeptide present in them is shown in Fig. 6. Baseplate proteins were identified by using phage 80a, which as mentioned above dissociates only the baseplate from the remainder of the virion when treated with EDTA, Tris–HCl, formamide.

Fig. 7 is a schematic summary allocating polypeptides seen on SDS–polyacrylamide gels to structures of serogroup B virions. The major polypeptide species from both head and tail-tube have a similar molecular weight, but these can be resolved on 8% acrylamide gels or by two-dimensional electrophoresis (Fig. 3). The additional low molecular weight polypeptide in phages 52A and 80a, referred to earlier (Fig. 2), appears to be a baseplate protein.

Dissociated virions from serogroup A could not be fractionated by differential centrifugation. Serogroup A virion heads are larger than serogroup B heads and probably have higher sedimentation coefficients, thus preventing their separation from the polymerized tail-tubes.

Fig. 4. Ultrastructural features of virions which differentiate serological and (within serogroup B) lytic groups. Virions from CsCl gradients were stained with ammonium molybdate. Phages representing each group are 84 (F), 81 (A), 187 (L), 52 (B I), 55 (B II) and 83A (B III). 'Full' head shown to the left of serogroup A virion illustrates the distortion of head shape seen in 'ghost'. Note the channel in the tail tube of the serogroup A and B II virions, which both have empty heads. Bar marker represents 50 nm.
Fig. 5. Polymerized tail-tubes. Phage 55 virions were disrupted, then the suspensions were diluted as described in the text. Under these conditions, single and partial tail-tubes polymerize. Bar marker represents 50 nm.

Fig. 6. Identification of tail-tube proteins. Phage 55 tail tubes were polymerized (see text and in Fig. 5). Polymerized tail-tubes were pelleted, then loaded onto 12% SDS–polyacrylamide gels for electrophoresis. The band of mol. wt. 64K may be a dimer of tail-tube protein (32K) formed as a result of residual formaldehyde in the formamide used for virion disruption.
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**DISCUSSION**

The phages of the International Typing Set have been collected over a period of about 40 years (Wentworth, 1963; Parker, 1983). Apart from the addition of new phages to the set, putative variants of existing types, with altered host range, have also been incorporated in it. The pedigrees of the current collection are not easily traced. The analysis of phage virion proteins and ultrastructure reported here provides a means, if not to untangle the history of these typing phages, then at least to identify molecular and possible genetic relationships among them.

On the basis of virion proteins, the typing set can be subdivided into four major groups (containing three or more members): group A, group B I, group B III, and group F III plus IV. Phages 55 and 71, which are in group B II, may be the basis for a fifth group. Phage 187 has a distinctive set of virion proteins, and this phage is also distinguishable from other phages of the set in that cells susceptible to phage 187 do not bind other typing phages, nor do host cells susceptible to the other typing phages bind phage 187 (Oeding, 1974). Cells susceptible to phage 187 contain N-acetylgalactosaminylribitol teichoic acid in their cell walls, rather than N-acetylglucosaminylribitol teichoic acid as found in non-susceptible cells (Karakawa & Kane, 1971; Oeding, 1974).

The number of polypeptides seen on SDS-polyacrylamide gels of the phage virions ranged between approximately seven and ten, at the amount of protein loaded in these experiments. Calculation of numbers of molecules of individual polypeptide species, assuming that the virions contain approximately equal amounts of protein and nucleic acid (Brown et al., 1972; Rosenblum & Tyrone, 1964), yields minimum values of approximately 30 molecules for observable individual virion proteins. Proteins present in smaller numbers of molecules per virion are detected on overloaded gels, but since these are not easily distinguished from minor contaminating host proteins the precise specification of minor virion polypeptides will have to await more detailed analysis, possibly by differential isotopic labelling of phage and host proteins.
proteins. By comparison with the coliphages λ and T1, which the aureophages resemble structurally and in genome size (Brown et al., 1972; Stewart et al., 1985), it would be anticipated that the aureophages contain 12 to 15 different protein species per virion, which is approximately what is seen on two-dimensional electrophoresis gels.

The major band on SDS–polyacrylamide gels of aureophage serogroup B virions contains two co-migrating polypeptide species. One of these species is the major head protein and the other is the tail-tube protein. The tail-tube is apparently assembled from a single polypeptide species. This is consistent with other phage virions (λ, T4) where a single protein is polymerized to form the tube. The major head protein accounts for 40 to 60% of the total virion protein according to densitometer analysis; in λ, the value is 57% (Buchwald et al., 1970). A minor head protein accounts for 5 to 10% of the total head protein, again as in λ.

The second major band on SDS–polyacrylamide gels of intact serogroup B virions does not appear in any of the head, tail-tube or baseplate fractions. Densitometer scans show that this protein accounts for 12 to 25% of the virion protein. A similar protein is found in λ: it does not appear in empty head or in tail preparations, and accounts for 19% of the total virion protein (Buchwald et al., 1970; Villarejo et al., 1967). It is believed to be the internal head protein (Georgopoulos et al., 1983).

Although not as precise in quantitative terms as DNA homology in the measurement of genetic relatedness, analysis of virion proteins is a simpler and faster method and thus permits a larger population to be sampled. Analysis of virion proteins in studies of phage molecular and genetic relationships may provide useful preliminary measures before embarking on more complex studies at the genome level. Polypeptide analysis, which permits rapid identification of phages at least to the serogroup level, may also enable more rapid epidemiological analysis of phage populations.

At the ultrastructural level all the phages of the International Typing Set are characterized by either isometric or elongate heads, with flexible tails, and a baseplate (except serogroup F virions, which may have a terminal knob). The dimensions of each of these structures varies, in some cases characteristically between sero- and lysogroups (Table 1). Thus, differences in tail length define the four serogroups, and within serogroup B, division into three lysogroups is possible on the same basis. Whether these differences are functionally related to differences between sero- and lysogroups will depend on examination of a wider range of aureophages, including recombinant or mutant phages.

Of the 24 phages examined, 23 have been studied by electron microscopy by other workers (Bradley, 1963, 1967; Rosenblum & Tyrone, 1964; Lapchine & Enjalbert, 1965; Brown et al., 1972; Ackermann et al., 1976; Krzywy et al., 1981). There are important discrepancies among these reports. Small differences in head dimensions are not considered significant since the distortion of heads is likely to vary from one preparation to another (Bradley, 1963). Small variations in tail length (up to 20 nm) are also not considered significant, because of distortion, shrinkage or expansion effects which may be related to differences in staining methods.

Krzywy et al. (1981) have summarized the differences seen in the earlier studies. Our observations are similar to those of Krzywy and co-workers, with the following significant exceptions. Phage 84 has a medium length tail (227 nm), not a short tail (155 nm), and is thus similar to other members of serogroup F. Phages 55 and 71 (group B II) have shorter tails (143 nm) than reported by Krzywy et al. (175 nm), consistent with the correlation of lytic group with tail length for serogroup B phages referred to previously (Table 1).

Variation among the typing phages has previously been encountered. Rosenblum & Tyrone (1964) reported four instances in which their morphological studies had disclosed a variation between serogroups: phages supposedly of groups B and F were in fact replaced by variants of the A serotype. Rippon (1956) found that variation from the F serotype to the B serotype could take place for phage 42D.

The variation in tail-tube length between different phage groups is an interesting phenomenon. The longest tails were those of serogroup A virions, which also have larger, elongate heads. Tolmach (1957) has suggested that the function of the phage tail is to bridge the zone of electrostatic repulsion between phage head and cell wall. It will be interesting to see...
whether the electrostatic charge on phage heads and on cells correlates with tail length (and host specificity).

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REFERENCES


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