ELECTRON MICROSCOPY AND SEROLOGY OF STAPHYLOCOCCUS PHAGES

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Williams and Fraser (1953) have described procedures for preparing highly purified suspensions of T-phages. Their electron microscope studies of such preparations have shown that the size and shape of frozen dried phages are quite different from the corresponding air dried specimens. We have, therefore, adopted their purification procedures and the freeze drying method of Williams (1953) in order to determine whether these methods are applicable for staphylococcal phages and at the same time to study their external morphology. We have found that frozen dried staphylococcal phage preparations differ markedly from air dried preparations.

Electron microscope studies of air dried preparations of staphylococcal bacteriophage E (Hotchin, 1954) and phage 3A (Farrant and Rountree, 1953) have been reported previously.

Six of the phages for the present study were derived from staphylococci of bovine origin, two of human origin. A study of the serology of the phages was also undertaken since this property is useful in their characterization (Adams, 1953). The eight phages tested fall into two serologically unrelated groups which differ morphologically. As would be expected, related phages have a similar appearance.

MATERIALS AND METHODS

 Cultures and phages. Micrococcus pyogenes cultures 10, 11, 12, 13, 14, and 15 were isolated from milk. These cultures are the propagating strains for phages S1, S2, S3, S4, S5, and S6, respectively. Phage S3 was isolated from culture 12, which behaved like the “frankly lysogenic” staphylococci described by Fisk (1942). Phage 42D and its indicator strain, 1363 (Blair and Carr, 1953), and phage 3A and its indicator strain, 284 (Jackson et al., 1954), are phages that are used for phage typing of staphylococci of human origin. Phages S1 and S2 were isolated by propagating phage 42D on the partially resistant cultures 10 and 11 respectively by method of Smith Williams (1948). Similarly, phages S4, S5, and S6 were isolated initially by propagating phage S3 on cultures 13, 14, and 15 respectively. Phage S3 has approximately the same efficiency of plating on cultures 10 and 13, and the same is true for phages S4 and S6 plated on culture 13. The other phages have maximum plating efficiency only on their propagating strains.

Standard phage methods were used for plaque counting and for determining the antiphage serum neutralization constants (Adams, 1950).

Nutrient broth (Albimi) and nutrient agar (Albimi) with the addition of 0.5 per cent sodium chloride were used for culturing and plating and for the propagation of phages S1, S2, S3, S5, 3A, and 42D. Phages S4, and S6 were best propagated in a yeast extract peptone, 0.5 per cent sodium chloride medium. Media for plaque counts and for propagating phages were adjusted to pH 8 with sodium hydroxide prior to autoclaving.

Five ml of about 10^8 bacteria per ml (18 hr culture) and 5 ml of about 10^9 phage particles per ml were added to 200 ml broth. Clear phage lysates (phage titer of 10^8 to 10^9 particles per ml) were usually obtained after 4 to 6 hr incubation at 30°C with the use of the rotary shaker. Use of the rotary shaker during incubation was required since static incubation produced varying phage titers of none to 10^9 particles per ml.

Phage purification by differential centrifugation (Williams and Fraser, 1953) was carried out as follows: Approximately 900 ml of clear phage lysate were centrifuged in a 21-rotor of a “spinco model L” ultracentrifuge for 90 min at 10,000 rpm. This was followed by alternate series of low and high speed centrifugations (5,000–8,000 rpm for 5 min for the low speed centrifugation and 10,000 rpm for 60 min in a 40-rotor for the
high speed centrifugation). Each low speed run was repeated twice and was then followed by a high speed centrifugation. Phage pellets were resuspended in a small volume of supernatant or in distilled water. The final phage pellet was resuspended in distilled water and centrifuged lightly.

Antiphage sera S5 and S6 were prepared in rabbits and their titer determined by the method of Rountree (1952).

Electron microscope preparations. The freeze drying method (Williams, 1953) was carried out as follows: Collodion or carbon covered electron microscope screens were mounted on brass blocks which were placed in a metal sublimation chamber cooled by a −70 °C dry ice methyl cellusolve bath. About 15 min (under vacuum to prevent condensation of moisture) were allowed for the brass blocks and the specimen screens to reach an equilibrium temperature with that of the sublimation chamber. The phage suspension was sprayed into the chamber with a high velocity spray gun directed toward the specimen screens. The chamber was sealed and evacuated with an oil forepump and a mercury diffusion pump. The sublimation was continued for about two hr with the temperature of the dry ice bath surrounding the sublimation chamber adjusted to about −50 °C.

Air dried specimens were sprayed with a high velocity spray gun or with a “vaponefrin nebulizer” onto collodion covered specimen screens. All phage specimens were shadowed with uranium and were examined in an RCA EMU 2 electron microscope.

<table>
<thead>
<tr>
<th>Phage</th>
<th>K S5 Anti-serum</th>
<th>K S6 Anti-serum</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A</td>
<td>0</td>
<td>14</td>
<td>A</td>
</tr>
<tr>
<td>S6</td>
<td>0</td>
<td>28</td>
<td>A</td>
</tr>
<tr>
<td>42D</td>
<td>17</td>
<td>0</td>
<td>B</td>
</tr>
<tr>
<td>S1</td>
<td>25</td>
<td>0</td>
<td>B</td>
</tr>
<tr>
<td>S2</td>
<td>20</td>
<td>0</td>
<td>B</td>
</tr>
<tr>
<td>S3</td>
<td>22</td>
<td>0</td>
<td>B</td>
</tr>
<tr>
<td>S4</td>
<td>21</td>
<td>0</td>
<td>B</td>
</tr>
<tr>
<td>S5</td>
<td>30</td>
<td>0</td>
<td>B</td>
</tr>
</tbody>
</table>

Figure 1. Electron micrograph of staphylococcus phage S6 prepared by air-drying. Magnified 40,000 X.

Figure 2. Phage S6 prepared by freeze drying. Magnified 40,000 X. Insert magnified 80,000 X.
RESULTS AND DISCUSSION

The serotypes and serum neutralization constants, tested against antiphage sera S5 and S6, are given in Table 1 for the eight phages we have tested. In each instance the homologous antiserum gave the highest K value, 30 for phage S5 and 28 for phage S6. It is apparent that phages S6 and 3A (serotype A) are related, and phages 42D, S1, S2, S3, S4, and S5 (serotype B) are related. The designation of the serotypes, A and B, was made on the basis of the nomenclature proposed by Rountree (1949), who classified phage 3A as serotype A and phage 42D as serotype B. We have thus designated the phages neutralized by the same antiphage serum that neutralized phage 3A as serotype A phages and likewise, the phages neutralized by the same antiphage serum that neutralized phage 42D as serotype B phages.

Phage S5 was originally isolated by adding phage S3 to culture 14 and phage S6 was isolated by adding phage S3 to culture 15. Since these two phages, S5 and S6, are of dissimilar serotypes it can be said that either the phage suspension of S3 was a mixture of more than one type of phage or an as yet to be determined phage host interaction took place when phage S3 was propagated on cultures other than its propagating strain 12, which has been found to be lysogenic.

Hotchin (1954) and Ralston and Krueger (1954) reported that the staphylococcus phages they purified by use of high speed centrifugation gave phage preparations in which many of the particles were fragmented. In agreement with these investigators we have found that high centrifugal rates result in considerable inactivation of the phage suspensions. This difficulty was largely overcome by limiting the rate of centrifugation to 10,000 rpm although this resulted in a lowered concentrating efficiency.

**Electron microscopy.** Initially eight air dried phage specimens were examined. They are morphologically of two general types; rather large particles with elliptically contoured, flattened heads and long tails (S6 and 3A, both of serotype A) and smaller particles with circular or polygonally shaped heads and long tails (S1, S2, S3, S4, 42D, and S5, all of serotype B). Thus, a correlation exists between the serotype and the morphology of the phages, serologically related phages being similar in size and shape.

The tails of both types of particles have a

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**Table 2**

<table>
<thead>
<tr>
<th>Dimensions of air-dried and frozen-dried staphylococcus phages S5 and S6 (dimensions in nm)</th>
<th>Phage S5</th>
<th>Phage S6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Air-dried</strong></td>
<td><strong>Frozen-dried</strong></td>
<td><strong>Air-dried</strong></td>
</tr>
<tr>
<td>Head</td>
<td>50 × 70</td>
<td>53 × 110</td>
</tr>
<tr>
<td>Tail</td>
<td>100 × 10</td>
<td>270 × 15</td>
</tr>
</tbody>
</table>

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**Figure 3.** Phage S5 prepared by air drying. Magnified 40,000 X.

**Figure 4.** Phages S5 by freeze drying. Magnified 40,000 X. Insert magnified 80,000 X.
pronounced terminal bob. The tails of the larger particles, 3A and S6, measure about 270 mμ in length, and their elliptical heads measure about 50 by 110 mμ. The tails of the smaller particles are about 160 mμ long and their heads are approximately 60 mμ in diameter. Representative electron micrographs of the two types of particles are shown in figures 1 and 3. The structure and the size of phage 3A are in accord with that reported by Farrant and Rountree (1953) for the same phage, and the general structure of the smaller particles is in accord with that reported by Hotchin (1954) for staphylococcus phage K and by Ralston and Krueger (1954) for phage P{	extsubscript{i}} variants.

One phage of each general type was selected for the freeze drying experiments. Phage S6, which is representative of the larger phages, is shown in figure 2. Phage S5, representative of the smaller phages, is shown in figure 4. The dimensions of the heads of the frozen dried phages are smaller than the dimensions of the corresponding phages air dried (table 2). The data presented in table 2 were obtained from electron micrographs of the same phage preparations of which half was used for the air dried and the other half for the frozen dried experiments. There is a noticeable difference in the structure of the phage heads; the frozen dried phages (figures 2 and 4) show the heads as generally polygonal in contour. Phage S5 (figure 4) often appears to be a regular hexagon in cross section and phage S6 (figure 2) appears to be an elongated hexagon in cross section. The shadows cast by the phage heads in figures 2 and 4 have linear sides in contrast to the more curvilinear shadows in figures 1 and 3. The size and shape of shadows in the figures 2 and 4 indicate that the frozen-dried phage heads are unflattened polyhedrons. The shadows cast by the particles in figures 1 and 3 suggest that the air-drying procedure caused a considerable and variable amount of distortion.

SUMMARY

Eight staphylococcus phages were examined in the electron microscope. The size and shape of frozen dried particles differed markedly from those of air dried particles. The phages within each group were shown to be similar morphologically, but the morphology of the two groups differed.

REFERENCES


