Purification, Fine Structure and Characterization of Temperate Phages from Drug-resistant Staphylococcus aureus

By Takeshi Saito, Takashi Osono, Mutsuhisa Inoue and Susumu Mitsuhashi

1Central Research Laboratories, Yamanouchi Pharmaceutical Co. Ltd., Itabashi, Tokyo and
2Laboratory of Resistance in Bacteria, School of Medicine, Gunma University, Maebashi, Japan

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SUMMARY

Three kinds of temperate Staphylococcus aureus phages were differentiated by serological, sedimentation and electron microscopic studies. Phage S1 had a long hexagonal head and a long tail, a density in Cs2SO4 of 1.365 g/ml and belonged to serological group A; phage S2 had a short hexagonal head and a short tail, a density of 1.385 to 1.392 g/ml and belonged to serological group B; phage S3 had a shape similar to phage S2 except for some minor differences, but had a density of 1.416 g/ml and belonged to serological group F. S. aureus MS27 was found to be singly lysogenic for S2. However, S. aureus MS3878 was a doubly lysogenic strain carrying S1 and S2 and S. aureus E169 was a triply lysogenic strain carrying S1, S2 and S3. All these temperate phages showed similarity in shape to typing phages belonging to the same serological group. The temperate S. aureus phages revealed the presence of a hexagonal baseplate with spikes. The burst sizes of phages S1, S2 and S3 were about 50, 110 and 120 respectively. The DNA from S1, S2 and S3 ranged from 29.4 to 30 megadaltons in size.

INTRODUCTION

Almost all strains of Staphylococcus aureus have been shown to be lysogenic, carrying prophages within the cell (Blair & Carr, 1961). Our genetic studies showed that drug-resistance genes were easily transduced among S. aureus cells by prophages (Mitsuhashi et al., 1965). This suggested a relationship between the intracellular state of the drug-resistance genes and temperate phages. We successfully induced and isolated temperate phages from drug-resistant S. aureus strains and examined them by electron microscopy (Saito et al., 1974). Later we obtained various temperate phages of a different serological type, and were able to propagate them easily on a non-lysogenic staphylococcal strain. Thus, we could show that some of these temperate phages transduced the drug-resistance genes.

Electron microscopy of S. aureus phages was first reported by Seto et al. (1956), and later by Bradley & Kay (1960), Bradley (1963) and Rosenblum & Tyrone (1964). Their fine structure has only been studied in detail recently (Brown et al., 1972). However, all these investigations were confined to an examination of typing phages.

In this paper we report the isolation, purification and characterization of temperate phages from three different drug-resistant clinical isolates of S. aureus.

METHODS

Bacterial strains. The strains used for induction of temperate phages were three multiple drug-resistant S. aureus strains: S. aureus MS27, resistant to tetracycline (TC), streptomycin...
(SM), sulphanilamide (SA) and penicillin (PC); *S. aureus* MS3878, resistant to TC, SM, chloramphenicol (CP), PC and showing inducible macrolide resistance by erythromycin (EMi); and *S. aureus* E169 resistant to TC, SM, SA and PC. *S. aureus* MS353 was used for propagation of temperate phages. This strain had been confirmed to be non-lysogenic by Kondo’s (1973) method.

**Media.** For the growth of bacteria and phage induction, the following liquid medium was used: Trypticase Soy Broth (BBL) 1%, Bacto-Brain Heart Infusion 0-5% (pH 7-4). Bacto-agar (0.3%) was added to provide a soft nutrient agar (soft NA) for plaque assay of the induced phages. A 0.05 m-tris–HCl buffer pH 7-4 containing 0-002 m-CaCl₂ (phage buffer) was used for dilution and storage of phage suspension.

**Phage induction, isolation and purification.** Cultures were induced in liquid using mitomycin C at concentrations of 0-1 to 0.5 µg/ml. Phage lysates were prepared in liquid media using *S. aureus* MS353 as host. High-titre phage stocks were prepared from crude lysates by differential centrifugation followed by density gradient centrifugation in Cs₂SO₄ (Szybalski & Szybalski, 1971). Fractions were collected and the refractive index, specific gravity and absorbance were measured. To prepare samples for electron microscopy the relevant fractions were centrifuged, the phage resuspended in phage buffer and then dialysed with the same buffer at 4 °C for 24 h.

**Electron microscopy of phages.** Negative staining was carried out with the procedure described by Nonomura et al. (1971). Uranyl acetate was prepared as 1-2% unbuffered solution.

**Serological typing.** Serological types of the temperate phages were determined by neutralization with antisera for A, B and F type phages (Delbrück, 1945; Adams, 1959; Rosenblum & Tyrone, 1964).

**One-step growth.** A 0.1 ml sample of the phage lysate and 0.9 ml of MS353 culture were added to 1 ml of nutrient broth containing 0.004 mM-KCN, and the mixture was incubated at 37 °C without aeration for 5 min. After addition of 0.1 ml vol. of anti-phage serum, the mixture was incubated at 37 °C for a further 10 min. The infected cells were centrifuged and resuspended in 10 ml of fresh nutrient broth and incubated at 37 °C with shaking. Samples (0.1 ml) were taken at appropriate time intervals, diluted with nutrient broth, and mixed with 2 ml of soft agar containing MS353. The mixtures were poured over NA plates and incubated at 37 °C.

**Electron microscopy of DNAs from purified phages.** Purified phage particles were incubated with 1 ml 0.5% SDS in buffer (10 mM-NaCl, 50 mM-tris, 10 mM-EDTA pH 7.6) at 37 °C for 5 min, and then for 30 min after the addition of 0.2 ml 1 mg/ml Pronase solution. The treated solution was extracted three times with an equal volume of buffer-saturated phenol. The aqueous phase was dialysed against SSC (15 mM-NaCl, 1.5 mM-sodium citrate) and used as the phage DNA sample. The DNA was examined in the electron microscope by the method of Gordon & Kleinschmidt (1968).

**RESULTS**

The serotype and density of temperate phages

Phages induced from *S. aureus* MS3878 produced two types of plaques. From small (0.5 mm diam.) plaques a phage designated S1 was isolated; from large (2 mm diam.) plaques a phage designated S2 was obtained. S1 was inactivated by antiserum to serotype A phages; S2 by that to serotype B phages. The density of the S1 phage was 1.36 g/ml, and that of S2 was 1.39 g/ml. Phage ghosts derived from S2 had a density of 1.31 g/ml. *S. aureus* MS27 was found to be singly lysogenic. The phage obtained from it was also an S2 type in that it
belonged to the serological group B; its density was 1.39 g/ml and its shape was similar to that of phage S2 obtained from S. aureus MS3878.

S. aureus E169 gave two sizes of plaques upon induction. From the small plaques an S1 type phage was obtained belonging to the serological group A. Its density was 1.37 g/ml. However, when the phage lysate from the large plaques was analysed by density gradient centrifugation, an extra, heavier band was observed along with the S2 phage band and that of the S2 ghosts. Phages obtained from this extra band were not neutralized with anti-B serum. The third temperate phage was named S3. This belonged to serological group F and had a density of 1.42 g/ml. The typical S2 phage was also isolated from the corresponding band. Its density was 1.39 g/ml. It should be noted that the density of the S2 phages was rather variable.

One-step growth

From one-step growth experiments, the latent period of the S1 phages derived from MS3878 and E169 was about 22 min and that of the S2 phages, including the one from MS27, was about 20 min. The average burst sizes of the S1 and S2 phages ranged from 45 to 50 and 106 to 110 respectively. The burst size of phage S3 was about 120 and its latent period was about 20 min.

Fine structure of S1 phages

Fig. 1 (a) shows an electron micrograph of the negatively stained S1 phage derived from S. aureus MS3878 as a representative of the S1 phages. Its head has the shape of a long hexagon, is 94.7 ± 1.0 nm in the long axis and 39.1 ± 0.3 nm in width. The flexible tail is 311 ± 20 nm long and is fitted with 68 repeated annulus-like structures suggesting turns of a spiral, each of which is 9.4 ± 0.6 nm wide and 2.9 ± 0.1 nm thick, and with two larger linking annuli 15.3 nm wide. At the distal end of the linking annuli there is a baseplate which has a knob-like appearance. A needle projecting from the centre of the knob is also seen in Fig. 1 (a). Neither of the S1 phages from MS3878 nor E169 have collar structures. Fig. 1 (b) shows broken ends of the tails of intact S1 phages and ghosts derived from MS3878. It should be noted that the central canal of the intact phage is larger (1.8 ± 0.3 nm) than that of the ghosts, and is uniformly packed with contents. After the S1 phage attached to the bacterial cell surface the diameter of the tail changed.

Fine structure of S2 phages

Fig. 1 (c) shows an electron micrograph of the negatively stained S2 phage from S. aureus MS27. The three S2 phages showed an almost similar structure but had minor differences in size and number of repeated units. The S2 phage from MS27 has a head with the shape of a regular hexagon and a flexible tail structure which extends from a vertex of this hexagon. The size of the head is 52.5 ± 5.0 nm wide. The tail is 160 ± 10 nm long, and is composed of 36 segments (i.e. repeated annulus-like structures) and has at its distal end two larger linking annuli and a baseplate. The repeated annuli are each 10.8 ± 0.2 nm wide and 3.0 ± 0.1 nm thick. They are placed perpendicular to the long axis of the tail. Each of the linking annuli consists of two rings 15.4 nm wide and 2.9 nm thick (amounting to a total thickness of 15.5 ± 0.3 nm). Unlike the other two S2 phages (those from MS3878 and E169), that from MS27 has no collar structure which connects the tail to the head at the proximal end.

Fine structure of S3 phages

Phage S3 has an almost similar size and structure to those of the S2 phages from MS3878 and E169. It has a hexagonal head 58.0 ± 0.5 nm wide and a tail 148 ± 10 nm long and 10.0
Fig. 1. (a) Electron micrograph of the S1 phage from *S. aureus* MS3878 negatively stained with uranyl acetate. The phage has a long hexagonal head and a very long flexible tail surrounded with 68 annulus-like structures. Larger linking annuli and a baseplate with a knob-like appearance are attached to the tail. A short needle projects from the baseplate. No collar is seen at the proximal end. (b) The S1 phage from *S. aureus* MS3878 showing broken ends of the tails. Broken ends of intact phages showing a hole filled with some material (T₁) and the broken end of a phage ghost (the head not shown) with an empty hole (T₂) are shown. (c) The S2 phage from *S. aureus* MS27. The phage has a hexagonally shaped head and a shorter flexible tail, is composed of 36 annulus-like structures, and fitted with two linking annuli at the distal end (L). A baseplate is attached distally to the linking annuli. The baseplate (B) of the intact S2 phage appears as a flat hexagon 20-4 nm wide, and on the bottom face has a
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It has a collar 15.4 nm wide at the proximal end of the tail as have the S2 phages from MS3878 and E169.

Structure of the baseplates of S2 phages

The shape of the baseplate of the S2 phages changed with release of DNA from the head. The baseplate of intact phages had a hexagonal shape 20.4 nm wide. This was smaller than that of the phage ghosts by 25 to 50% just like the T4 phage (Simon & Anderson, 1967). It should be noted that a needle was seen penetrating the baseplate before DNA in the head was released (Fig. 1 c). The first observable change was rounding out of the spikes to form a six-point star (Fig. 1 e). Fig. 1 (d) shows the change of the baseplate after the release of DNA. The baseplate has taken the form of a disc-like structure with a central hole surrounded by a clockwise distorted star (viewed from end). A central canal through the tail which resulted from the release of DNA is shown clearly. Spikes projecting out of the baseplate were also clearly seen in the side view. It was noted that the linking annuli and the baseplate were connected by a fibre-like structure (results not shown).

DNA size

Electron microscopy of the intact DNAs extracted from purified phages revealed linear molecules. The mol. wt. of the DNA from the S1 and S2 phages from MS3878 were 29.4 × 10^6 and 30.4 × 10^6 and the phages S1, S2 and S3 from E169 were 29.4 × 10^6, 30.2 × 10^6, and 30.0 × 10^6 respectively.

DISCUSSION

Results of our studies on the fine structure of temperate phages from S. aureus are summarized in Fig. 2. These studies revealed that our temperate phages were similar in gross morphology to the typing phages belonging to the same serological group. That is, the S1 phages belonging to serotype A had a long hexagonal head and a long tail characteristic of the typing phage of serotype A. The S2 phages belonging to serotype B were smaller, with a hexagonal head and a shorter tail like the serotype B typing phage. But in two out of three S2 phages we found a collar structure connecting the tail with the head; the third one from MS27 had no collar. This difference in morphology suggests a need for subdivision of the S2 phages in spite of their gross morphological and serological similarity.

Fine structures of the typing phage or serotype F are not yet known. Our S3 phage belonged to serotype F and had a shape similar to that of the S2 phages which had a collar. Serotype F phages are associated with gentamicin resistance (de Saxe & Porthouse, 1979) and lysogenic conversion has been observed (Kondo, 1973). This aspect requires further investigation. The S3 phage had the highest density but electron microscopy revealed that its head and DNA were not particularly large. A similar case was reported on mutants of the T5 phage, which showed high density in CsCl but had no extra DNA (Saigo, 1978). Differences in structural material may be responsible.

A morphological change in the tail after release of the DNA was found. This differed from that of the T-even phages (Simon & Anderson, 1967). On release of DNA no contraction of the tail occurred in these staphylococcal phages. Instead, the baseplate of the S2 phages changed to a disc with a clockwise distorted star.

disc-like structure (D) and a short needle (N) penetrating its centre. (d) A ghost of the S2 phage from S. aureus MS3878. This well-resolved micrograph reveals the clockwise distorted star structure of the baseplate and its disc with a central hole. A collar is also clearly seen (arrow). (e) The S2 phage from S. aureus E169. A tail end of an intact phage viewed from the end. The baseplate has a six-point star structure. All bar markers represent 100 nm.
We had already reported that S1 phages are capable of transducing tetracycline, erythromycin and penicillin resistances at an extremely high frequency (Inoue et al., 1976).

S2 phages were found to transduce drug-resistance plasmids at a frequency of about $10^{-5}$ to $10^{-6}$. By contrast, the transduction frequency of plasmids with S1 phages was about $10^{-8}$ to $10^{-10}$. Phage S3 also had the transducing ability of plasmid DNA. However, the relationship between the serological group and transducing ability has not been fully elucidated yet.

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**REFERENCES**


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