Determination of Genome Size of Selected Typing Bacteriophages of *Staphylococcus aureus*¹

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The molecular weights of six representative typing bacteriophages were determined by sucrose gradient centrifugation. Genome size appeared to be related to the size of the phage head.

As part of an earlier study (7) to define certain physical and biochemical properties of the staphylococcal typing bacteriophages, we have determined the molecular weights of the genome of six different bacteriophages. The viruses selected represent an overview of the lytic and serological groups presently employed. Although we have used these data in an examination of the biological relationships used for classification, we are aware that this study does not represent all 21 phages and, therefore, may be incomplete in this regard. Nevertheless, high degrees of correlation have been encountered among members of the same serological groups (7), suggesting a broader significance.

Staphylococcal typing bacteriophages 47, 81, 71, 80, 77, and 187 were propagated, purified, and typed as reported earlier (7).

Molecular weight and sedimentation coefficient (corrected to viscosity of water at 20°C) (s₂₀,ₚ) values for the phenol-extracted (13), purified staphylococcal deoxyribonucleic acids (DNA) were determined by using 5 to 20% linear sucrose gradients prepared in ½ in by 2 in (ca. 1.3 by 5.1 cm) nitrocellulose tubes. In such gradients (3, 5, 6) the s₂₀,ₚ values varied linearly with time. The buffer system employed was 10 mM tris(hydroxymethyl)aminomethane, pH 7.6, containing 1 M NaCl and 1 mM ethylenediaminetetraacetic acid (12). Coliphage T7 DNA (the gift of Melvin Center, Kansas State University, Division of Biology) was used as a sedimentation and molecular weight marker. A molecular weight of 26 × 10⁶, and an s₂₀,ₚ value of 32, was assumed for coliphage T7 DNA (12). Molecular weights of the staphylococcal phage genomes were calculated by using the relationship derived by Burgh and Hershey (3). Control experiments showed that sedimentation variations due to concentration effects (3) were negligible with the quantities of DNA used in this study (0.5 to 5 μg of DNA per gradient). In general, the phage DNAs sedimented as narrow, homogeneous bands. There was no evidence of contamination with degraded host DNA which would have sedimented slowly as broad bands. Rapidly sedimenting material was occasionally observed at the bottom of the gradients and was attributed to aggregation of phage DNA (3, 4) or to wall effects (3). It was possible to obtain consistent and reproducible sharp, symmetrical peaks by using this method of zonal centrifugation. The calculation of molecular weight and s₂₀,ₚ values of the staphylococcal phage genomes studied were within a standard deviation of ±3%. The range for molecular weight calculations was 3 × 10⁶ or less (Table 1). The sedimentation values and molecular weight data for the six bacteriophages studied are presented in Table 1. Phage 47 and phage 81 possessed a large genome with a molecular weight of 39 × 10⁶ to 40 × 10⁶ and an s₂₀,ₚ value of 37. The apparent discrepancy between the s₂₀,ₚ values (both 37) and the molecular weights (40 × 10⁶ for phage 47 DNA, 39 × 10⁶ for phage 81 DNA) occurred because the relationship used to calculate molecular weights (3) is more sensitive to small experimental differences than is the relationship used to determine sedimentation rates.

Phage 71 DNA was smaller than either phage 47 DNA or phage 81 DNA, having a molecular weight of 29 × 10⁶ and an s₂₀,ₚ value of 33. Phage 80, the other serological group B phage studied (lytic group I), possessed a genome that was smaller than the serological group A genomes. Phage 80 DNA had a molecular weight

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of $31 \times 10^6$ and an $s_{20,w}$ value of 34 (Table 1). The values obtained for the serological group B phages (71 and 80) are similar to those reported by Brown et al. (2) for staphylococcal phage P11-M15, a serological group B phage not in the typing series. The genome of P11-M15 had a molecular weight of $32.7 \times 10^6$ and an $s_{20,w}$ value of 35.1 (13).

The DNA of phage 77 DNA (serological group F, lytic group III) was smaller than the DNAs from the serological group A phage (47 and 81), but larger than the DNAs from the serological group B phages (71 and 80). The molecular weight of phage 77 was $37 \times 10^6$ and the $s_{20,w}$ value was 36.

Phage 187 DNA was about the same size as the DNAs from the serological group B phages, having a molecular weight of $30 \times 10^6$ and an $s_{20,w}$ value of 34.

The data in Table 1 correlated well with staphylococcal phage morphological studies. Serological group A phages are morphologically the largest staphylococcal viruses, having oblong heads varying from 50 by 110 nm for phage S6 (11) to 60 by 80 nm for phage 3B (1). The tails of all group A phages are about 300 nm long (1, 10, 11). Correspondingly, the serological group A phages studied in this investigation, phages 47 and 81, possessed the largest genomes (Table 1).

In contrast, serological group B phages are smaller than group A phages (1, 9–11) (symmetrical heads, about 50 nm in diameter, and tails about 150 nm long), and, as expected, the genomes of the serological group B phages, 71 and 80, were smaller than the genomes of the group A phages.

Phage 187, the serological group L phage studied, has a slightly larger head (60 nm) and a slightly longer tail (170 nm) than phage 71 (head diameter of 50 nm, tail length of 150 nm) (1), but the data (Table 1) show that the genomes of phage 187 and phage 71 are about equal in size ($30 \times 10^6$ daltons for the genome of phage 187, $29 \times 10^6$ daltons for the genome of phage 71). Unfortunately, complete comparison of the serological L phage 187 with the B phages investigated in this study is not possible, since morphological data are not available for phage 80.

Phage 77, the serological group F phage studied, has head dimensions about the size of serological group B and L phages (55 nm) but a considerably longer tail (220 nm) (1). Thus, it is intermediate in size between the smaller serological groups B and L and the larger serological group A phages. The size of the phage 77 genome is also intermediate, being larger than the serological group B and L phages, but smaller than the serological group A phages (Table 1).

Although only six phages were studied, the work presented here points out further similarities among the serological groups and is consistent with our earlier findings (7).

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