Buoyant Density Analysis of Staphylococcal Bacteriophage 80 Transducing Particles

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Received for publication 29 July 1969

Densities of *pase*, *tet*, and *ol* transducing particles were indistinguishable, and about $0.002 \text{ g} \times \text{cm}^{-3}$ less dense than the plaque-forming units (PFU). The densities of the PFU and *nov* transducing particles were indistinguishable, the PFU having a density of $1.507 \text{ g} \times \text{cm}^{-3}$.

Dowell and Rosenblum (1) reported that phage particles responsible for the transduction of novobiocin resistance in lysates of staphylococcal phage C had a buoyant density which was indistinguishable from that of the plaque-forming units (PFU) of this phage. However, the analyses of generalized transducing phages P1 and P22 have revealed some discrete but characteristic differences between the buoyant densities of transducing particles and infectious particles (2, 8, 9, 12).

This study is concerned with the buoyant density distribution of infectious and transducing particles obtained by propagating staphylococcal phage 80 on strain U9 (*pase*, *tet*, *ol*, *nov*) of *Staphylococcus aureus* (5). This strain of *S. aureus* produces penicillinase (*pase*), and is resistant to tetracyclines (*tet*), oleandomycin (*ol*) and novobiocin (*nov*). Phage 80 particles obtained by propagation on this strain were pelleted at $110,000 \times g$ and 20 C for 30 min, and were gently suspended in P and D broth (5) to titers of 2×10^{11} to 5×10^{11} PFU/ml. A 45% (w/w) solution of CsCl was prepared by mixing technical grade CsCl (American Potash and Chemical Corp., Los Angeles, Calif.) with equal volumes of phage lysate and 2X suspension medium (13). Solutions prepared in this manner had a density of about $1.51 \text{ g} \times \text{cm}^{-3}$ as determined by refractometry. Portions (2 ml) of this solution, overlaid with mineral oil, were centrifuged in a Spinco SW56 rotor at 25,000 rev/min and 20 C for 22 hr. Immediately after centrifugation, the bottom of each tube was pierced (6), and successive one-drop fractions were collected, each into a separate tube containing 2 ml of P and D broth. In addition, the contents of one gradient were collected in toto and were assayed for infectious particles. The recovery of PFU

from the density gradients was invariably about 50% of the recovery from identical CsCl-phage solutions which had not been centrifuged.

The one-drop fractions were each examined for PFU and transducing particle content. Strain 152 of *S. aureus* (5) was used as the recipient in all transduction experiments. The transduction procedure used has been described (5), except that the transduction suspensions were prepared to contain 10^{-3} M CaCl_2 . The presence of CaCl_2 greatly improved the transduction frequencies of all markers (Table 1).

Figure 1 presents the buoyant density distributions of the PFU and the transducing particles which were obtained. The peak fraction (relative number = 100) for PFU contained 1.4×10^{10} infectious particles. The peak fractions for transducing activities contained 3410 *pase*, 1320 *nov*, 41,600 *tet*, and 44,600 *ol* transducing particles. The *nov* transducing particles exhibited a buoyant density which was indistinguishable from the buoyant density of the PFU. However, the *pase*, *tet*, and *ol* transducing particles were two fractions less dense than the PFU. From the slope of the density gradient, it was calculated that the buoyant densities of these transducing particles are about $0.002 \text{ g} \times \text{cm}^{-3}$ less than the densities of the PFU and *nov* transducing particles.

Thermal induction of *Escherichia coli* K-12 (λ *ind*⁻ CI857), kindly supplied by A. D. Kaiser, Stanford University Medical Center, provided a sample of phage λ . When phage λ was centrifuged together with phage 80 in a Spinco model E analytical ultracentrifuge according to Weigle et al. (13), the phages could not be resolved into two ultraviolet-absorbing bands. Therefore, phage λ , with a buoyant density of $1.508 \text{ g} \times \text{cm}^{-3}$ (13), was used as the reference to determine the buoyant density of staphylococcal phage 81.

TABLE 1. Effect of the presence of 10^{-3} M CaCl_2 in the transduction suspension on the transduction frequency

Marker	Transduction frequency ^a	
	Without CaCl_2	With CaCl_2
<i>pase</i>	230	1,940
<i>tet</i>	4,230	36,720
<i>nov</i>	540	4,760
<i>ol</i>	1,880	21,100

^a Recombinants per 10^8 plaque-forming units.

FIG. 1. CsCl gradient of PFU and transducing particles of phage 80. Relative number is the percentage of the activity recovered in peak fraction (relative activity = 100).

Using phage 81 as the density reference ($1.476 \text{ g} \times \text{cm}^{-3}$), phage 80 exhibited a buoyant density of $1.507 \text{ g} \times \text{cm}^{-3}$. This value is within the range of densities for group B staphylococcal phages (7).

As is true with the P1-*E. coli* and P22-*Salmonella typhimurium* systems (2, 11), the densities of the deoxyribonucleic acids (DNA) of the phage and donor bacterium of the phage 80-*S. aureus* system are different. Szybalski (10) has reported

that staphylococcal phage 80 DNA has a density of $1.697 \text{ g} \times \text{cm}^{-3}$ relative to *E. coli* DNA ($1.710 \text{ g} \times \text{cm}^{-3}$). Using the same reference DNA in the present study, DNA extracted from strain U9 (*pase*, *tet*, *ol*, *nov*) exhibited a density of $1.693 \text{ g} \times \text{cm}^{-3}$ when centrifuged according to the method of Mandel et al. (3). Assuming that phage 80 consists of about equal parts of protein and DNA, the difference between the DNA of phage and donor strain ($0.004 \text{ g} \times \text{cm}^{-3}$) correlates well with the difference of $0.002 \text{ g} \times \text{cm}^{-3}$ between infectious particles and the *pase*, *tet*, and *ol* transducing particles of phage 80.

There are at least two explanations for the greater density of the *nov* transducing particles compared to the *pase*, *tet*, and *ol* transducing particles. Either the *nov* transducing particles consistently contain a greater amount of DNA, or this DNA represents a portion of the donor genome which has a density which is higher than the average. Ozeki and Ikeda (4) have proposed a "wrapping choice" mechanism for the formation of generalized transducing particles in which infectious and transducing particles both contain identical amounts of DNA. Considering this mechanism and the relationship between the densities of phage and host DNA and the particles which contain them, it is concluded that the *nov* transducing particles probably contain DNA which is of "normal" length, but of higher than normal density.

This investigation was supported by grant GB-5568 from the National Science Foundation and by Public Health Service grant AI-06647 from the National Institute of Allergy and Infectious Diseases.

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