BACTERIOPHAGE-P22 DERIVATIVE WITH HIGH DENSITY

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Summary

Bacteriophage P22Cm21 was differentiated in some characters from the original phage P22. The buoyant density in CsCl solution of phage P22Cm21 was higher than that of phage P22 by as much as 0.007 g per cm^3. The possible biological implication involved in this higher density is discussed.

Introduction

P22Cm phages, plaque-forming recombinants between P22 and P1Cm, were isolated from a P22 phage lysate grown on P1Cm-lysogenic Salmonella typhimurium and transduced the Cm‡ marker by lysogenization at high frequencies upon single infection. These P22Cm phages can be classified into two groups by the physiological characteristics (1). One of these, designated Group I P22Cm, makes small plaques on Salmonella strains. The titer of Group I phage lysates usually obtained was very high, more than 10^{11} PFU per ml. The other group, designated Group II, makes very tiny plaques on the indicator strains. The titer of Group II lysates was not so high, 1 - 10 x 10^9 PFU per ml. No structual differences could be found among phages P22Cm1 (Group I P22Cm), P22Cm21 (Group II P22Cm) and wild-type P22 under an electron microscope (Suzuki and Mise, unpublished observations). In this report, we describe that

‡ Abbreviations used: Cm, chloramphenicol; Cm^r, resistance to chloramphenicol; PFU, plaque-forming unit.

phage P22Cm21 shows higher density in CsCl solution than the original phage P22.

Materials and Methods

Bacterial strains, bacteriophages, media and chemicals used were as described in the previous paper (1).

Transduction generally followed the method of Lennox (2).

CsCl density-gradient centrifugation. A 0.1-ml aliquot of a P22Cm lysate (about 5×10^8 PFU per ml) and the same amount of a P22 lysate (about 2×10^8 PFU per ml) were suspended in 5.2 ml of CsCl-0.1 M Tris buffer, pH 7.0. The refractive index of the solution was 1.3834. After centrifugation for 18 hr at 30,000 rpm and 5°C in Beckman SW50·1 rotor, about 55 fractions were collected. Each fraction was assayed for plaque-forming activities and for abilities to transduce the Cm marker. Plaque formers in a P22Cm lysate and those in a P22 lysate were differentiated from the form of plaques on the indicator strain LT2 4415. The former made small plaques, while the latter did ringed plaques.

Sucrose density-gradient centrifugation. A 0.1 ml of phage solution containing P22Cm21 (3×10^8 PFU per ml) and P22 (1×10^8 PFU per ml) were layered on a 5.0-ml gradient of 5 - 20 % sucrose in standard saline citrate. After centrifugation of the gradient for 28 min at 20,000 rpm and 15°C, 77 fractions were collected from the botton of a tube and assayed for plaque-forming activities and Cm^r-transducing activities.

Results

In spite of their plaque-forming activities, Group II P22Cm phages had several characteristics which were more or less related to the characteristics of defective phages. For example, phage P22Cm21 made very tiny plaques on a Salmonella strain 4415. The burst size and the latent period of phage P22Cm21 were much smaller and longer than those of P22 or P22Cm1, a representative Group I phage (Fig. 1). Phage P22Cm21 was inactivated at a high temperature at much higher rate than phage P22 or P22Cm1 (Fig. 2). These results may suggest the possibility that the constituents of a P22Cm21 particles are somewhat different from those of a P22 particle.

To demonstrate this possibility, each of phages P22Cm1 and P22Cm21 was submitted to CsCl density-gradient centrifugation in

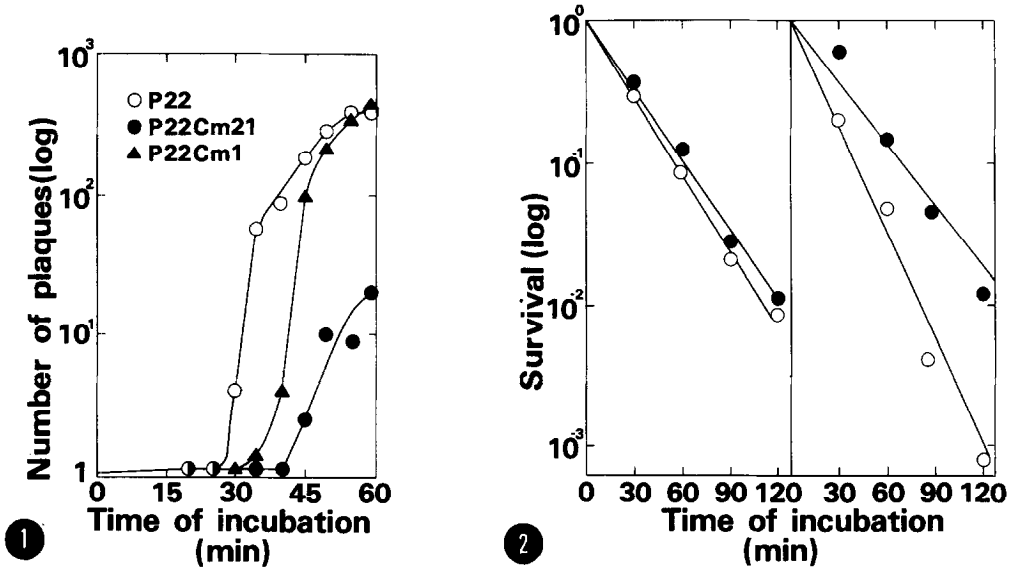
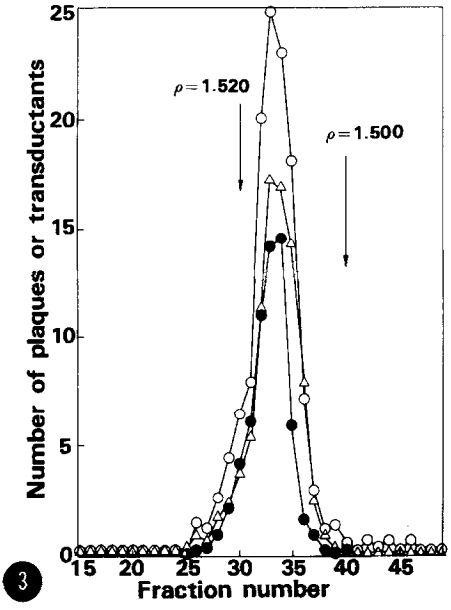


Fig 1. One step growth curves of phages P22, P22Cm1 and P22Cm21. The host strain used was Salmonella typhimurium 4415.

Fig 2. Heat inactivation of phages P22, P22Cm1 and P22Cm21. Phages were diluted 100-fold in prewarmed 0.01 M Tris buffer, pH 7.4, and incubated at 73.5C. Left, P22(●) and P22Cm1(o); right, P22(●) and P22Cm21 (o). Strain 4415 was used as the indicator for titration of plaque-forming particles.

reference to phage P22. As shown in Fig. 3, the density of the Cm^r-transducing particles as well as the plaque-forming ones in a P22Cm1 lysate agreed with that of phage P22. In contrast with phage P22Cm1, the density of phage P22Cm21 particles, either transducing or plaque-forming, was higher than that of phage P22 (Fig. 4). We repeated the experiments four times to obtain consistently the same results; the density of phage P22Cm21 was higher than that of phage P22 by 0.006 - 0.008 g per cm^3.

Phage P22Cm21 was then centrifuged in the 5 - 20 % sucrose gradient. As shown in Fig. 5, the plaque-forming particles of phage P22Cm21 sedimented slightly faster than those of phage P22.



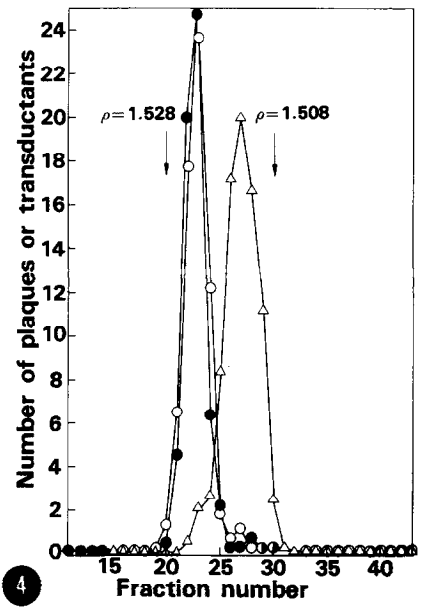


Fig 3. CsCl equilibrium density-gradient centrifugation of P22Cml lysate. A mixture of P22Cml with P22 was centrifuged in CsCl solution as described in Materials and Methods. The total number of fractions was 57. (o), Plaque-forming activity of P22Cml; (•), Cm^r-transducing activity of P22Cml; (△), plaque-forming activity of P22. One unit on the ordinate corresponds to 5×10^6 PFU per ml for (o), 2×10^5 PFU per ml for (△) and 5×10^4 transductants per ml for (•).

Fig 4. CsCl equilibrium density-gradient centrifugation of P22Cm21 lysate. The total number of fractions was 55. (o), Plaque-forming activity of P22Cm21; (•), Cm^r-transducing activity of P22Cm21; (△), plaque-forming activity of P22. One unit on the ordinate corresponds to 1×10^7 PFU per ml for (o), 5×10^6 PFU per ml for (△) and 1×10^5 transductants per ml for (•).

The Cm^r-transducing particles and the plaque-forming ones of phage P22Cm21 co-sedimented in the gradient (data not shown).

Discussion

Our experiments presented here clearly indicated that the density of phage P22Cm21 was higher than that of wild-type phage P22. If the difference in density between phages P22Cm21 and P22 is dependent upon the length of DNA alone, phage P22Cm21 would

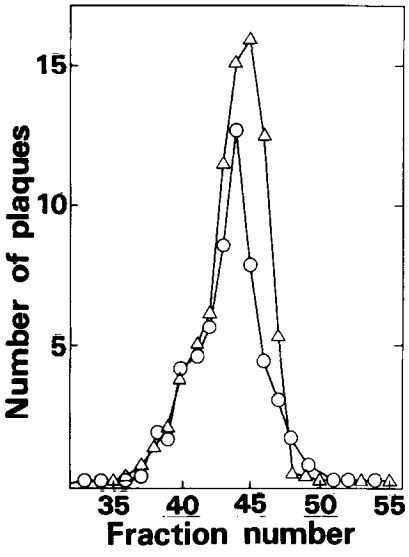


Fig 5. Sucrose-gradient centrifugation of P22Cm21 lysate. The total number of fraction was 77. (o), Plaque-forming activity of P22Cm21; (△), plaque-forming activity of P22.

be expected to contain 5 to 8 % excess DNA on the basis of the formula of Weigle et al (3).

Phage P22 DNA is known to be encapusilated into the particle by the "headful" mechanism (4), in which the length of the DNA of the particle is determined by the amount of DNA that can be contained in a phage head (5, 6). From this model, we can expect that the length of all P22 DNA's is the same in the particle and that the densities of all the particles are also the same, unless the constituents of viral proteins or the density of the DNA per se are changed among P22 phages.

Unlike in the P22-Salmonella system, several density mutants have been reported by various workers in the lambda-Escherichia coli system (3, 7, 8). The amount of DNA in these mutants is different from that in the original phage lambda. For example,

phage lambda b2 contains 18 % less DNA than the wild type, while lambda p4 contains 9 % excess DNA. The results herein described are well explained by the action of lambda-specific endonuclease.

Further experiments are now in progress to determine whether the higher density of phage P22Cm21 is due to the higher content of DNA or lower content of protein constituents in the phage particles. Preliminary experiments with isolated phage DNA suggest that the latter case is more probable, though the former can not be fully denied.

Acknowledgments

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