Antiserum Inactivation of Electrophoretically Purified M13 Diploid Virions: Model for the F-Specific Filamentous Bacteriophages

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Antiserum inactivation experiments were carried out on electrophoretically purified diploid virions from a cross between two complementing amber mutants of phage M13. The total (homozygous plus heterozygous) diploid population, assayed on a permissive host where only one genome is needed for plaque formation, was inactivated at the same rate as haploids. Heterozygous diploids, assayed on a nonpermissive host, where both genomes are needed for plaque formation, were twice as sensitive as haploids and the total diploid population. These results have led us to propose a model for serum inactivation of the F-specific filamentous phages. According to this model, phage-neutralizing antibodies attach anywhere along the length of the phage and allow the phage to penetrate only up to the first bound antibody molecule.

Genetically heterozygous particles of F-specific filamentous (Ff) bacteriophages were first reported by Notani and Zinder (*Bacteriol. Proc.*, p. 140, 1964) and more extensively by Scott and Zinder (13), in the progeny stock of mixed infections between complementing f1 amber mutants. These heterozygotes were detected by their ability to form plaques in a lawn of nonpermissive indicator bacteria. The high frequency at which they were found, 1 to 5%, excluded the possibility of their being wild-type recombinants or revertants. Moreover, their plaques were found to contain both parental phages.

Since f1 heterozygotes could be distinguished biologically from the parental phages by the type of host cell on which they would form plaques, many of their properties could be studied without separating them from other virions in the same preparation. It was found, for instance, that when nonpurified stocks of crosses were inactivated by UV-light irradiation or by anti-f1 serum, the heterozygous phages, assayed on a nonpermissive (*su*⁻) host, were twice as sensitive as the total population assayed on permissive (*su*⁺) indicator bacteria. From the serum inactivation results, the heterozygous particles were believed to contain twice as many adsorption sites for antibody molecules

as the parental phages. It was suggested, therefore, that antibody molecules neutralized f1 virions by binding anywhere along the length of the phage particles. From these and other results, Scott and Zinder (13) postulated that f1 heterozygotes were double-length particles containing two separate DNA molecules enclosed in the same coat protein and that they were produced in the infected cells and not by subsequent aggregation.

The hypothesis of Scott and Zinder (13) suggesting that heterozygous f1 phages were double-length particles was supported by Caro and Schnös (3) who observed by electron microscopy that polymeric virions of f1 adsorbed to the tip of F pili, just like single-length virions. Scott and Zinder (13) also reported the finding, by electron microscopy, that 8% of the virions in the total population were twice as long as the majority of the phages.

Double-length M13 virions were partially purified by ion-exchange chromatography of progeny stocks from genetic crosses, and many of their physical and biological properties were studied (11). It was determined that these phages contain two separate, circular, single-stranded DNA molecules. The terms "haploid" and "diploid" were used to denote the single- and the double-length virions containing one and two DNA molecules, respectively. It was also found that up to 50% of the diploid population consisted of virions containing two genetically different DNA molecules, one of each parental type. These, therefore, are heterozy-

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gous diploids, or "hets," and correspond to the heterozygotes of f1 (G. W. Notani and N. D. Zinder, *Bacteriol. Proc.*, p. 140, 1964; 13). Phages in the remainder of the diploid population contain duplicate copies of one or the other parental phage genome and are, therefore, homozygous. Salivar et al. (11) also showed that diploid particles are not merely aggregates of single-length phages occurring in vitro, but that they are actually produced as such by infected cells, as proposed by Scott and Zinder (13).

Although the partially purified diploids were tested for inactivation by UV-light irradiation (11), they were not subjected to antiserum inactivation. Therefore, the behavior of the total diploid population compared with that of hets was not determined.

In a companion paper (1), we report the complete purification of haploid and diploid M13 virions by electrophoresis in dilute polyacrylamide gels. It was, therefore, decided to attempt antiserum inactivation of such purified virions. In order to include an internal biological control in each experiment, it was decided to perform inactivations on electrophoretically purified virions from the progeny stock of the cross between amber mutants *am5-H3* and *am6-H1*, which both form clear plaques on K37. Then an unelectrophoresed stock of amber mutant *am2-H2*, which forms turbid plaques, could be added as the control. Results of these experiments have led to a model for antibody inactivation of Ff bacteriophages.

MATERIALS AND METHODS

Strains, media, and procedures. The bacteriophages, bacterial host strains, and standard media and procedures for M13 have been described previously (7, 8, 11, 12).

Virions from the progeny of the cross between M13 amber mutants *am5-H3* and *am6-H1* were subjected to electrophoresis in 2.5% polyacrylamide gels as described in the companion paper (1). Phages from the haploid and the diploid peaks were used for antiserum inactivation experiments.

Anti-M13 serum was prepared by William O. Salivar (12) and kept in the frozen state until used.

Inactivation of M13 virions. Serum inactivation experiments were carried out at room temperature and with highly diluted (10^6 -fold) antiserum in order to slow down the kinetics of the reaction and allow sufficient time for samplings and platings. Survivors were assayed on K37 (*su*⁺) and K38 (*su*⁻) indicator bacteria.

RESULTS

Scott and Zinder (13) showed that f1 heterozygotes are twice as sensitive to antiserum as the total population in a nonpurified progeny stock of a cross. We wanted to find out how

electrophoretically purified diploids, homozygous as well as heterozygous, would behave compared with the total population. Serum inactivation of purified haploid virions was also followed.

Results, in the form of survival curves, are shown in Fig. 1. The single-hit kinetics of these curves show that in all cases, a single antibody molecule can inactivate a phage particle.

As shown in Fig. 1A, the electrophoretically purified haploid virions were inactivated at the same rate as the unelectrophoresed *am2-H2* control.

Serum inactivation of purified diploid virions is shown in Fig. 1B. As expected from the results of Scott and Zinder (13), hets were inactivated twice as rapidly as the haploid *am2-H2* control. Surprisingly, though, the total diploid population, assayed on K37, was inactivated at the same rate as the haploid control.

DISCUSSION

The fact that diploids which can only form plaques if they inject two genomes are inactivated at twice the rate of diploids which only need to inject one genome has led us to a model for antibody inactivation of M13 which we believe is applicable to all Ff bacteriophages. This model, illustrated in Fig. 2, postulates the

FIG. 1. Antiserum inactivation of M13 virions purified by polyacrylamide gel electrophoresis. A, Haploid virions; B, diploid virions. The electrophoretically purified haploid and diploid virions from the progeny stock of the cross between amber mutants *am5-H3* and *am6-H1* were subjected to inactivation by anti-M13 serum, as described in Materials and Methods. The phage suspensions were diluted down to 4×10^4 PFU/ml in buffer (0.05 M Tris-chloride, pH 8.90), and mixed with an equal volume of a stock of M13 amber mutant *am2-H2* (a turbid plaque-former) of the same titer. To 0.9 ml of phage suspension was added 0.1 ml of a 10^6 -fold dilution of antiserum. Incubation of the mixture was carried out at room temperature, samples were taken at intervals, diluted if needed in buffer, and assayed for surviving plaque-formers on both K37 and K38, for the total population and for heterozygotes, respectively.

FIG. 2. Model for antibody inactivation of Ff bacteriophages. This model is based on results shown in Fig. 1 and on postulates mentioned in the text.

following: (i) coat proteins and genomes of Ff bacteriophages are intimately connected; (ii) genomes of diploid and multiploid particles are arranged end-to-end within Ff virions and penetrate sequentially into the bacterial host cell; (iii) phage-neutralizing antibody molecules bind anywhere along the length of an Ff virion, as suggested by Scott and Zinder (13), and have no detectable effect on phage attachment; and (iv) phage penetration begins from an end of the virion and is blocked upon reaching a bound antibody molecule.

According to this model, therefore, if an antibody molecule binds to the proximal half of a diploid virion, i.e., that closest to the adsorption site, no genome can enter the cell and the phage is inactivated: it cannot give rise to a plaque, even on a permissive host. On the other hand, if the antibody molecule binds to the distal half of a diploid virion, the first genome will still be able to infect a host cell and will multiply if the host is permissive. Under such conditions, however, a heterozygote is prevented from giving rise to a plaque on a nonpermissive host because the second genome needed cannot enter into the cell.

Consequently, even though diploids are twice as long as haploids, their sensitive target is only the proximal half, equivalent to the length of a haploid virion, in assays on a permissive host, and their rate of inactivation remains that of haploids. In the case of heterozygous diploids assayed on a nonpermissive host, however, the sensitive target is twice that of a haploid virion and they are inactivated twice as rapidly as haploids.

Virions from a triploid-rich fraction (1) have also been subjected to serum inactivation (J. Beaudoin, Ph.D. thesis, University of Wis., 1970). Results (not shown here) indicate that

the total triploid-rich fraction is inactivated at the same rate as the haploid *am2*-H2 control. This can be explained in light of the model proposed here. Phages in the triploid-rich fraction, whatever their lengths, can give rise to a plaque if they can first adsorb to host cells and then inject one genome. If an antibody molecule binds to the proximal part of the phage particle, however, even the first genome cannot enter. Therefore, the sensitive site is the same size as that of a haploid, whatever the length of the phage particle, and all phages are inactivated at the same rate.

Although the exact mode of entry of filamentous phages is still unknown, the above model for inactivation by anti-phage serum is compatible with either Brinton's "F-pili conduction model" (2), in which the phage somehow penetrates into the host cell through the pilus; or Marvin's "F-pili retraction model" (6), in which the phage is somehow pulled into the cell with the pilus. Also, this model for antibody inactivation of Ff phages does not rule either in favor of or against the polarity of phage adsorption, although there is some published evidence suggesting that Ff bacteriophages are indeed polar (4, 5, 9, 10).

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