Pilus-specific, Lipid-containing Bacteriophages PR4 and PR772: Comparison of Physical Characteristics of Genomes

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SUMMARY

The genomes of pilus-specific, lipid-containing phages PR4 and PR772 were studied electron microscopically. An identical mol. wt. of $10^9 \times 10^6$ was obtained. The genomes are unique (non-permuted) and have cohesive ends. From the similarities in size and denaturation maps of the genomes and failure to demonstrate non-homology in heteroduplexes, reported morphological ambiguities were clarified. The known serological difference between the phages could not be related to non-homology of their genomes. It is concluded that phages PR4 and PR772 are the same phage.

INTRODUCTION

Pilus-specific phages PR3, PR4 (Stanisich, 1974; Bradley & Rutherford, 1975) and PR772 (Coetzee et al. 1979) have recently been studied extensively. Phages PR3 and PR4 have been compared with respect to their morphology, buoyant density, sensitivity to chloroform, inactivation by antiserum, adsorption to host cells, host range, growth on infected bacteria, efficiency of plating and plaque morphology. The only difference observed was in their growth characteristics, reflected in plaque morphology. Hence they can be viewed as plaque-type mutants of the same virus (Bradley & Rutherford, 1975). Attachment of PR4 to pili specified by plasmids of the P-I, N and W incompatibility groups has been demonstrated (Bradley & Rutherford, 1975; Bradley, 1976; Bradley & Cohen, 1977).

Following the isolation of phage PR772 on Proteus mirabilis strain PM5006 nal-r(R772), this plasmic-specific phage was compared with phage PR4. The phages proved to be identical in the above respects, except for differences in morphology and inactivation by antiserum (Coetzee et al. 1979). Although they are hexagonal and exhibit a thick inner layer, presumably lipid, the diam. of PR4 has been reported as 65 nm v. 53±3 nm for that of PR772. Phage PR4 reportedly has a tail of variable length whereas PR772 has no tail. Antisera produced with phage PR772 in three rabbits showed inactivation constants ($K$) of 6.76, 4.86 and 8.32 per min and when used against PR4, $K$-values of 0.68, 0.20 and 0.34 per min, respectively, were obtained. The difference in $K$-value is consistently one order in magnitude (Coetzee et al. 1979).

In order to elucidate the above ambiguities, the genomes of phages PR4 and PR772 were studied electron microscopically. We report here on mol. wt., homoduplex, heteroduplex and denaturation mapping analyses.

METHODS

General. Escherichia coli J62-1 (R772) was used as host for phages PR4 and PR772, all from the laboratory stocks of this Unit. The methods described by Adams (1956) and Coetzee (1974) were used.
Purification of phage and nucleic acid extraction. These were done as described by Coetzee et al. (1979) and Olsen et al. (1974).

DNA microscopy. The methods of Coetzee & Pretorius (1979), involving benzylalkyldimethylammonium chloride (BAC) were used. Molecules were photographed at an instrumental magnification of 6800, by means of a Philips EM 300 electron microscope.

Data processing. Photographic negatives were projected in a rear-view fashion and were traced by means of an Electronic Graphics Calculator (Numonics Corporation, Lansdale, Pennsylvania) interfaced with a Hewlett Packard 9825A calculator. Computer simulations of molecules were plotted and integrated to yield denaturation maps.

Mol. wt. determinations. These were done electron microscopically relative to the length (14.8 μm) of the Proteus mirabilis phage 5006 M genome of mol. wt. 30.7 × 10^6 (Pretorius & Coetzee, 1979).

RESULTS

Electron microscopic appearance of genomes

Linear and circular forms were observed. In both cases multiple lengths of an apparent monomer were encountered. Incubation at 55 °C for 10 min in the presence of 5% formaldehyde converted all circles and concatenates to the linear monomeric length. This suggests that both genomes are unique and have cohesive ends. Examples of such genomes are known, e.g. λ (Davidson & Szybalski, 1971) and the lambdoid phages 21, 80, 82, 424 and 434 (Yamagishi et al. 1965; Baldwin et al. 1966; Kaiser & Wu, 1968).

Mol. wt. of genomes

The lengths of PR4 molecules were measured relative to the length of the phage 5006 M genome on the same photographic plates. The average value for the length ratio PR4: 5006 M was found to be 0.35±0.02 (s.d.). Assuming the mol. wt. of phage 5006 M to be 30.7 × 10^6 (Pretorius & Coetzee, 1979) the mol. wt. of phage PR4 is 10.9 × 10^6. Similar measurements on PR772 genomes yielded values of 0.35±0.01 (s.d.) and 10.9 × 10^6, respectively. There is no significant difference in mol. wt. between the phage genomes.

Partial denaturation

Partially denatured molecules of PR4 and PR772 DNA were monomeric. Computer simulations of such molecules resulted in Fig. 1(a), 2(a), respectively. Sixty molecules of each phage facilitated construction of denaturation maps (Fig 1b, 2b).

These results confirm the uniqueness of both phage genomes. The similarity of the denaturation maps (Fig. 1b, 2b) is evident. They do not reveal any significant difference in broad characteristics between the genomes of phages PR4 and PR772, as minor discrepancies may be attributed to a difference in the average extent of denaturation (22% for PR4 and 31% for PR772).

Duplex analyses

Homoduplex preparations of PR4 and PR772 DNA contained high yields of double stranded molecules. These were indistinguishable from the untreated preparations and this supports the above suggestions as to the uniqueness of the genomes.

Heteroduplexes of PR4 and PR772 DNA molecules likewise yielded double-stranded molecules. These were scrutinized for regions of non-homology but none was observed.
Comparison of PR4 and PR772 genomes

Fig. 1. (a) Computer simulations of 60 partially denatured PR4 DNA molecules, (b) denaturation map deduced from the data in (a).
Fig. 2. (a) Computer simulations of 60 partially denatured PR772 DNA molecules, (b) denaturation map deduced from the data in (a).
Comparison of PR4 and PR772 genomes

DISCUSSION

The above results indicate that the genomes of phages PR4 and PR772 are unique, that they have cohesive ends and identical mol. wt. Within the limits of electron microscopic detectability, non-homology between the phage genomes could not be demonstrated.

It has been shown (Vollenweider et al. 1975) that when the benzylalkyldimethylammonium chloride monolayer technique of the present experiments is used, denaturation can be detected during unstacking of base pairs, prior to physical strand separation. This emphasizes the high resolution that can be expected in partial denaturation and heteroduplex experiments. Non-homology can be resolved to about 200 base pairs (unpublished observations). Consequently, if the reported morphological and serological differences between phages PR4 and PR772 are related to differences in their respective genomes, such differences have to be small. We suggest that as for morphology, this is not likely.

Firstly, the discrepancy in head size, 65 nm for PR4 v. 53 nm for PR772, manifests itself in the outer layer, which consists of protein (Bradley & Rutherford, 1975; Coetzee et al. 1979). In view of the similarity in genome size and phage buoyant density (Coetzee et al. 1979) it follows that the difference in diam. necessitates an almost twofold difference in phage mass. This has to be accounted for by proteins in the capsid. However, the geometric and physico-chemical limitations on the number and character of the constituents of icosahedrally symmetrical phage heads would necessitate the presence of proteins in the large capsid that differ from those in the small capsid (Caspar & Klug, 1962). Conceivably the genetic sequence for such proteins would exceed 200 base pairs, which would have been detectable in our heteroduplex experiments. We conclude that the reported size difference between these phages should be viewed with caution.

Secondly, it is well known that a number of phage gene products are required for tail synthesis and assembly. These genes may occupy a considerable portion of the phage genome, e.g. phages λ (Szybalski & Herzkowitz, 1971), T2, T4 and T6 (Kim & Davidson, 1974). The present findings on size and sequence homology of the genomes of phages PR4 and PR772 lead to the conclusion that no difference exists between these phages as for the existence of tails. However, in view of the relatively small size of their genomes, we favour the notion of Coetzee et al. (1979) that tail-like structures observed on some PR772 particles are in fact broken-off pili to which the phages had attached. This would account for the variable lengths of tail-like structures on PR4, as observed by Bradley & Rutherford (1975).

Antigenic determinants are thought to be small and consist of 5 to 20 amino acid residues (Davis et al. 1973). Consequently, if an altered protein is to account for the observed difference in serum neutralization rate constant between phages PR4 and PR772, it would imply a nucleotide sequence difference < 60 nucleotide pairs – clearly beyond our electron microscopic detectability.

In view of the large body of evidence regarding the similarity of phages PR3, PR4 and PR772, we conclude that they are in fact the same phage. The only (serological) difference between phages PR4 and PR772 may be attributed to a minor difference in the amino acid sequence of otherwise identical capsid proteins.

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REFERENCES


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