Biological characterization of the lytic cycle of actinophage φA7 in \textit{Streptomyces antibioticus}

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Received 23 June 1991
Accepted 27 June 1991

Key words: Actinophage φA7; \textit{Streptomyces antibioticus}; Adsorption; One-step growth curve

1. SUMMARY

Some basic parameters of the lytic development of phage φA7 in \textit{Streptomyces antibioticus} are described. One-step growth experiments demonstrated that at 28°C φA7 has a latent period of about 60 min and an exponential growth period of about 35 min. The average burst size ranged from 70–100 plaque forming units per infected cell. At the same temperature 50% of the virions were adsorbed to germ tubes of \textit{S. antibioticus} in about 10 min. This corresponds to an adsorption constant of $6.5 \times 10^{-10}$ ml/min. The phage was unable to adsorb the host at other stages of the life cycle (spores or mycelium). Divalent cations are not required for φA7 stability but Ca$^{2+}$ proved to be essential for adsorption and also for a later stage of the vegetative development of the phage.

2. INTRODUCTION

Bacteria of the genus \textit{Streptomyces} are common inhabitants of the soil. They have a complex life cycle (involving sporulation, spore germination and mycelial growth) and are of commercial interest because of their capacity to produce a wide range of antibiotics. Actinophage (phage that infect actinomycetes) are of increasing interest in the study of \textit{Streptomyces} molecular biology (see ref. 1, for a review). They are abundant in nature and can be readily isolated from soil samples. From this source we have obtained several actinophages which infect \textit{Streptomyces antibioticus}, a producer of the macrolide antibiotic oleandomycin [2]. One of these phage, φA7, was characterized in some detail and data on its general and molecular biology have been published [2,3]. The phage is temperate, showing a wide host range. Like most \textit{Streptomyces} phage it has a polyhedral head (52 nm in diameter) and a long, non-contractile tail (102 nm in length), ending in a base plate. The phage genome is a 46.7-kb double-stranded-DNA molecule with cohesive ends. Two regions which are not required for
plaque formation were located in the restriction map: a 1.2-kb region at about 18 kb from the conventional right-hand end of the linear-DNA molecule, and a larger segment of about 6.2 kb, close to the left-hand end. The DNA of the phage can integrate into the chromosome of several Streptomyces strains to form stable lysogens. Integration occurs through recombination between attachment sites on the phage (attP) and host (attB) genomes. The \( \phi A7 \) att\( P \) site has been located near the left-hand end of the phage DNA, close to the largest dispensable region.

In order to increase our knowledge of the general biology of \( \phi A7 \) and its interaction with the host, we have established in the present work the basic parameters of the lytic cycle as well as the optimal conditions for the propagation and preservation of the phage.

3. MATERIALS AND METHODS

3.1. Bacteriophages, bacterial strains and general techniques

\( \phi A7 \), originally described in [2], was the phage used throughout this work and \( S. \) antibioticus ATCC 11891 was the host for the propagation of the phage. Methods for propagation, assay and purification of the phage, and for culture of Streptomyces were essentially as in [4], but GAE medium [5] was used for growth of \( S. \) antibioticus.

3.2. Adsorption

Adsorption rates of \( \phi A7 \) to young germ tubes of \( S. \) antibioticus were determined by free-phage titration [6]. Nutrient broth (Oxoid) containing 1% glucose was inoculated with \( 10^8 \) spores/ml, and incubated at 28°C with shaking, until short germ-tubes were formed (about 6 h). Germinated spores were mixed with phage at a multiplicity of infection of 0.1 and then \( \text{Ca(NO}_3\text{)}_2 \) was added to a final concentration of 100 mM. The adsorption mixture was kept at 28°C with shaking. Samples were withdrawn every 5 or 10 min, centrifuged at 12000 rev/min and free phage determined on \( S. \) antibioticus top layers. The adsorption constant (K) was calculated according to Adams [6]. The same method was used to assay adsorption at other stages of the developmental cycle of the host, but spores were incubated for different times (see below) before the addition of the phage.

3.3. One-step growth experiment

The experiment was carried out essentially as described by Dowding [7]. Following a 20 min period of adsorption (see above) the infected culture was filtered through 0.45-\( \mu \)m filters (Millipore) and washed three times with 5 ml of nutrient broth to eliminate free phage. Cells collected on the filter were resuspended by shaking in 25 ml of nutrient broth with 100 mM \( \text{Ca}^{2+} \) (first infected culture, containing \( 10^7 \) cells/ml) and an 0.5-ml sample was further diluted with 25 ml of the same medium (second infected culture, with \( 2 \times 10^5 \) cells/ml). Both cultures were incubated at 28°C with shaking and at 5–10 min intervals samples were removed and immediately assayed for plaque-forming units (pfu).

4. RESULTS AND DISCUSSION

4.1. Plating and preservation conditions

The effect of \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) on the efficiency of plating (eop) of \( \phi A7 \) was assayed by plaque formation on lawns of \( S. \) antibioticus. Appropriate dilutions of the phage lysate were plated on nutrient agar containing a range of MgCl\(_2\) and/or Ca(NO\(_3\))\(_2\) concentrations. The results obtained are summarized in Table 1. In the absence of both \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \), no plaques were detected (eop < \( 10^{-4} \)). \( \text{Ca}^{2+} \) was shown to be essential for phage propagation. The size and number of plaques increased with increasing concentrations of this cation, with an optimum of 100 mM. This seems unusually high, as the optimum for other actinophages is in the range of 4–25 mM [4]. At higher concentrations (data not shown) the outline of the plaques was difficult to determine because the medium became turbid and very soft. Mg\(^{2+}\) could not replace Ca\(^{2+}\). Moreover, in combination with the optimal Ca\(^{2+}\) concentration, a range of Mg\(^{2+}\) concentrations had no effect on the plating efficiency of \( \phi A7 \). However, at suboptimal concentrations of Ca\(^{2+}\) addition of Mg\(^{2+}\) led to some improvement in the eop.
Table 1
Influence of divalent cations (Ca$^{2+}$ and Mg$^{2+}$) on φA7 propagation (Several concentrations of Ca$^{2+}$ (A) and Mg$^{2+}$ (B) were tested in the absence of the other cation. 100 mM Ca$^{2+}$ was selected as the optimal concentration. Further improvement in the eop was not achieved by addition of Mg$^{2+}$ together with the optimal Ca$^{2+}$ concentration).

<table>
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<tr>
<th></th>
<th>Ca(NO$_3$)$_2$</th>
<th>eop $^a$</th>
<th>MgSO$_4$</th>
<th>eop $^b$</th>
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<td>10</td>
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<td>2.9</td>
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<td>5.5</td>
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<tr>
<td>100</td>
<td>9.0</td>
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$^a$ Final concentration (mM).
$^b$ The eop was calculated with respect to a control supplemented with 8 mM Ca(NO$_3$)$_2$ and 10 mM MgSO$_4$.
$^c$ Very small plaques.

The influence of temperature on the eop of φA7 on S. antibioticus was also tested. The phage produced plaques at 28 °C, 34 °C and 37 °C, with similar eop. However, the plaque size decreased with increasing temperature, probably due to the faster growth of the host. No plaques were formed at 45 °C (eop < 10$^{-6}$) although the host was able to grow at this temperature.

Therefore, in accordance with these results, 100 mM Ca$^{2+}$ and 28 °C were used in the experiments described below.

Since divalent cations are known to enhance phage stability, the effect of Ca$^{2+}$ on survival of φA7 was analyzed. Dilutions (10$^{-1}$–10$^{-5}$) of phage in nutrient broth with and without added Ca(NO$_3$)$_2$ (100 mM) were stored at 4 °C. At one month intervals samples from each dilution were removed and plated on S. antibioticus top layers. After at least 6 months storage, titres did not fall even in the absence of Ca$^{2+}$. Stability of φA7 in the absence of divalent cations is in agreement with the relatively high resistance of the phage to treatment with chelating agents [3].

4.2. Adsorption

The adsorption of φA7 to young germ tubes of S. antibioticus was studied as described in MATERIALS AND METHODS. Plotting the percentage of unadsorbed phage against time (Fig. 1) the curve indicated that 50% of adsorption had already occurred within about 10 min. The titre of free phage continued to decrease for 50 min and, before 60 min it increased sharply, indicating lysis of infected cells and release of newly synthesized virions. Thus, the latent period established for φA7 by adsorption experiments is close to 1 h. Ca$^{2+}$ proved to be essential for adsorption since there was no loss of free phage during a period of 2 h in the absence of this cation.

Adsorption of φA7 at other stages of the life cycle of S. antibioticus was also studied. Results indicated that φA7 particles only adsorb to germ tubes of varying length (6–10 h of incubation at 28 °C). Thus, the phage was unable to adsorb to dormant, dark and swollen spores (0–5 h) or to young and old mycelium (12, 24, 36 and 48 h). In all these cases recovery of free phage was always higher than 90%.

The fact that φA7 adsorption is limited to germ tubes is noteworthy. Many actinophages, including PK-66 [8], VP11 [7], Pal 6 [9] and the well-characterized φC31 [10,11], can also adsorb to young mycelium. In this connection, it is relevant to point out that the limits between germ tubes and mycelial growth are somehow artificial (10–12 h of incubation at 28 °C were arbitrarily
set as the limit in this paper). Furthermore, little is known about the changes which might occur at the cell surface in the transition from one stage to another. Therefore it would be interesting to identify the host receptor for φA7, accessible only in germinated spores. Whatever the nature of this receptor, it does not seem to be available in all Streptomyces species. For example, the phage is unable to infect *S. lividans* due to adsorption failure (L.H. Diaz, unpublished results).

Adsorption rate constants of φA7 to germ tubes of *S. antibioticus* were low, ranging between $1.0 \times 10^{-9}$ ml/min (5 min after contact) and $2.3 \times 10^{-10}$ ml/min (50 min after contact). This correlates well with the values reported for most actinophage-actinomycete systems (around $10^{-9}$ ml/min) [12], which are, in general, an order of magnitude lower than those for coliphage [6]. It has been suggested that low rates of adsorption for actinophages are due to asynchrony of the developmental cycle of the host and the consequent variation in competence of the population for phage infection [12]. This may also be the case for φA7.

### 4.3. One-step growth curve

The multiplication curve of actinophage φA7 is shown in Fig. 2. It was determined by measuring the pfu/ml in serial samples of infected germ tubes of *S. antibioticus*. The curve gave a value of 60 min for the latent period, close to the one obtained in adsorption experiments. The rise period was 35 min, and the burst size varied between 70–100 pfu per infected cell. Release of virions did not occur (less than 10 pfu per infected cell) when Ca$^{2+}$ was absent from the dilution medium (added to avoid secondary infections, see Materials and Methods). Ca$^{2+}$ therefore is not only required for adsorption but also for a later stage of the intracellular cycle of φA7 in *S. antibioticus*.

**ACKNOWLEDGEMENTS**

L.A.D. was the recipient of a grant from the Plan de Formación del Personal Investigador of the Ministerio de Educación y Ciencia (Spain). The work was supported by Grant PB-85-0403 from the CAICYT (Spain). The authors are grateful to Dr. D.G. Swan and Dr. K.F. Chater for helpful comments on the manuscript.

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