Polyamines in Bacteriophage φW-14 and in φW-14-infected

Pseudomonas acidovorans

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SUMMARY

Bacteriophage φW-14 is sensitive to osmotic shock. It contains sufficient free putrescine, 2-hydroxyputrescine and spermidine to neutralize about 15% of the DNA phosphates. The α-putrescinythymine residues of the DNA could neutralize a further 25% of the phosphates. Label is transferred from ornithine to the α-putrescinyl residues of φW-14 DNA. The rates of polyamine synthesis in Pseudomonas acidovorans are increased by φW-14 infection.

The polyamines putrescine and spermidine are widespread in bacteria (Cohen 1971; Bachrach, 1973). They occur also in several Escherichia coli bacteriophages (Ames, Dubin & Rosenthal, 1958; Ames & Dubin, 1960; Dion & Cohen, 1972; Fukuma & Cohen, 1973; Bachrach, Fischer & Klein, 1975). The capsids of the T-even phages behave like semi-permeable membranes: the particles are osmotically fragile, and they retain their polyamines when dialysed against divalent cations such as Mg²⁺ (Ames & Dubin, 1960). However, in the osmotically stable phages T3, T5 and φX174, the polyamines are largely replaced by the divalent cations (Ames & Dubin, 1960; Bachrach et al. 1975).

Polyamines are synthesized by Escherichia coli after infection with T4 (Dion & Cohen, 1971), T5 and φX174 (Bachrach et al. 1975) and the RNA phage R17 (Fukuma & Cohen, 1973). With T5 and φX174 there is an increase in ornithine decarboxylase (ODCase) activity after infection (Bachrach et al. 1975).

Bacteriophage φW-14 is structurally similar to the T-even phages (Kropinski & Warren 1970); however, it is unusual in that its DNA contains the hypermodified pyrimidine, α-putrescynylthymine (Kropinski, Bose & Warren, 1973). Its host, Pseudomonas acidovorans, contains much 2-hydroxyputrescine in addition to putrescine and spermidine (Karrer, Bose & Warren, 1973). Unlike E. coli, P. acidovorans forms these polyamines from ornithine but not from arginine and is impermeable to putrescine (Karrer & Warren, 1974). This means that the polyamines of P. acidovorans can be labelled by use of radioactive ornithine or glutamate, but not by arginine or putrescine.

Now we have determined the free polyamine content of φW-14 and have investigated, polyamine synthesis in φW-14 infected bacteria.

Pseudomonas acidovorans 29 was grown on casamino acids-mannitol (CAA-M; Kropinski et al. 1973) for the preparation and titration of phage stocks and on tris-HCl-casamino acids-succinate (TCS; Lewis et al. 1975) for the experiments. Growth was followed turbidimetrically. Cultures were always infected with a m.o.i. of 10 at a density of 3 × 10⁸ cells/ml.

The preparation and titration of phage stocks have been described (Kropinski et al. 1973). Phage for analysis was purified by the method of Lewis et al. (1975), then dialysed exhaustively against dimethylglutarate buffer (Ames & Dubin, 1960).

Phages were subjected to osmotic shock by suspending approximately equal numbers of p.f.u. of φW-14 and T4 in TN buffer (0.01 M-tris-HCl–0.15 M-NaCl, pH 7.4) and in TN buffer containing 5 M-NaCl; after 20 h at room temperature, the TN suspension was diluted.
Table 1. Polyamine content of φW-14

<table>
<thead>
<tr>
<th>Preparation</th>
<th>p.f.u./ml</th>
<th>OD&lt;sub&gt;260&lt;/sub&gt; (10&lt;sup&gt;12&lt;/sup&gt; p.f.u.)</th>
<th>μmol phosphate per OD&lt;sub&gt;260&lt;/sub&gt; unit</th>
<th>Polyamines (nmol/μmol phosphate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2·0 x 10&lt;sup&gt;11&lt;/sup&gt;</td>
<td>32·5</td>
<td>0·093</td>
<td>Putrescine: 30, Spermidine: 22·5, 2-hydroxyputrescine: 12</td>
</tr>
<tr>
<td>2</td>
<td>2·5 x 10&lt;sup&gt;11&lt;/sup&gt;</td>
<td>38·8</td>
<td>0·094</td>
<td>Putrescine: 28·6, Spermidine: 17·4, 2-hydroxyputrescine: 9·8</td>
</tr>
</tbody>
</table>

100-fold in TN; the TN/5 mM-NaCl suspension was diluted by a factor of 10<sup>8</sup> in distilled water and in 3 M-KCl. After 30 min at room temperature, the surviving phage were assayed for p.f.u. on P. acidovorans 29 and on E. coli B.

The incorporation of radioactive putrescine by phage-infected cells was determined as described previously for uninfected cells (Karrer & Warren, 1974). Phage DNA was labelled by adding the appropriate radioactive compound (0·25 μCi and 20 μg/ml) to cultures at a cell density of 1·5 x 10<sup>8</sup>/ml. After infection and lysis, the labelled phage was purified, and its DNA extracted and purified by the methods of Lewis et al. (1975). The DNA was hydrolysed in 6 N-HCl (Kropinski et al. 1973) and the bases separated by chromatography on cellulose sheets (Eastman Chromagram sheets 6064, without fluorescent indicator) using isopropanol-concentrated HCl-water (65:17:18, v/v/v; Bendich, 1957). The u.v. absorbing areas were excised from the sheet and transferred to scintillation vials for radioassay.

The phosphate content of phage was determined colorimetrically after ashing (Ames & Dubin, 1960). DNA was measured by the diphenylamine method (Burton, 1968). Radioactivity was determined by adding 5 ml of scintillation fluid (see Lewis et al. 1975) to each vial and measuring the disintegrations in an Isocap 300 liquid scintillation spectrometer.

Putrescine dihydrochloride and spermidine trihydrochloride were from Calbiochem (San Diego, California); dansyl chloride was from Nutritional Biochemical Co. (Cleveland, Ohio); all radiochemicals were from New England Nuclear (Montreal, P.Q.). Dr C. Hurwitz kindly provided a sample of 2-hydroxyputrescine.

TCS medium is used routinely now in our work. Since our previous estimates of the polyamine levels in P. acidovorans were made on cultures growing in M29 medium (Karrer et al. 1973), cultures growing in both media were compared. There were about 15 nmol of polyamines per 3 x 10<sup>8</sup> cells in both media, but there was more putrescine (12 versus 6 nmol) and less 2-hydroxyputrescine (3 versus 6 nmol) in cells growing in TCS.

Dilution of the TN/5 mM-NaCl suspension with distilled water destroyed 98 and 99 % of the p.f.u. of φW-14 and T4, respectively. When the initial dilution was made with 3 M-KCl all the φW-14 survived, but 40 % of the T4 p.f.u. were destroyed.

φW-14 contained free polyamines. Spermidine, putrescine and 2-hydroxyputrescine were present in the molar ratios 0·7:1·0:0·4 (Table 1). Since the ratios for the uninfected host were 0·12:1·0:0·26, the phage contained relatively more spermidine and less 2-hydroxyputrescine than P. acidovorans. However, φW-14 infected cells took up no more putrescine than uninfected cells (see Fig. 1c in Karrar & Warren, 1974).

φW-14 DNA was labelled by 5-<sup>14</sup>C-ornithine; all the radioactivity (1680 ct/min in the area cut from the chromatogram) was associated with the α-putrescylthymine of the DNA. φW-14 DNA was not labelled by <sup>14</sup>C-U-arginine nor by <sup>14</sup>C-<sup>3</sup>-ornithine. During the lytic cycle, ornithine was incorporated into pronase resistant material which, after phenol extraction, banded at a density of 1·666 g/ml in a CsCl gradient. Therefore, it was assumed
that the experiment measured the incorporation of ornithine into φW-14 DNA. Incorporation started 20 min after infection, at which time there was also a dramatic increase in DNA synthesis (Fig. 1a).

The polyamine content of cells fluctuated in a characteristic manner after φW-14 infection (Fig. 1b). Between 5 and 20 min after infection, all three polyamines decreased markedly. After about 20 min, at which time DNA synthesis resumed (Fig. 1a), all three polyamines increased markedly, but after 35 min they decreased again. Between 20 and 40 min, the polyamine content of infected cells increased at a faster rate than that of uninfected cells (Fig. 1b).

φW-14, like T4, is sensitive to osmotic shock, and it contains free polyamines. In T4, the polyamine content is sufficient to neutralize about 40% of the negative charges on the DNA phosphate (Ames & Dubin, 1960). Significantly, although φW-14 contains free polyamines sufficient to neutralize only some 15% of the phosphate charges, the α-putrescinylnthymine residues could neutralize a further 25% of the charges.

P. acidovorans is impermeable to putrescine. φW-14 infection increases the rate of polyamine synthesis but it does not render the cells permeable to putrescine. The fluctuation in polyamine levels after infection may be related to leakage after phage adsorption and injection. Polyamines leak from E. coli after infection with T4 (Ferroluzi-Ames & Ames, 1965; Shalitin, 1968).

P. acidovorans does not convert arginine to polyamines (Karrer & Warren, 1974). φW-14 infection does not lead to the appearance of a phage-specific pathway for the conversion of
arginine to putrescine, since arginine does not label φW-14 DNA. The failure of 14C-1-
ornithine to label φW-14 DNA suggests, but does not prove, that the putrescine moiety of α-
putrescinythymine is derived from putrescine. However, the carboxyl group might be
eliminated after formation of the secondary amine at the δ-nitrogen. We are attempting to
isolate a putrescine permeable mutant to clarify this point. The labelling of φW-14 DNA
by ornithine is very useful for experiments on the biosynthesis of α-
putrescinythymine and its distribution in φW-14 DNA.

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