Biophysical properties of Mycobacteriophages

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SUMMARY

Biophysical characterization of two mycobacteriophages (Phlei phage and Butyricum phage) was carried out. Biophysical parameters obtained were: (i) buoyant densities in CsCl of 1.51 g/ml for both phages; (ii) sedimentation coefficient of 490 S and 410 S; (iii) DNA content of 42 and 34 % and (iv) mol. wt. calculated by electron microscopic dimensions to be $123 \times 10^6$ and $116 \times 10^6$ for Butyricum and Phlei phage, respectively.

Mycobacteriophages have been studied by microbiological and morphological methods (Redmond, 1963; Takeya & Amako, 1964; Kölbl, 1970). Their nucleic acid and protein components have been characterized in a few reports (Tokunaga & Sellers, 1964; Sellers & Tokunaga, 1966; Castenuovo, Giuliani & Giuliani, 1969; Castenuovo, Bellezza & Borelli, 1970; Castenuovo, 1970), but a detailed biophysical investigation has not been carried out.

In the present paper we have investigated biophysical properties of two mycobacteriophage strains: mycobacteriophages butyricum and phlei (abbreviated to Butyricum and Phlei phages respectively isolated and characterized by Vandra (1965).

Host bacteria (Mycobacterium phlei and M. butyricum) were grown by shaking in Sauton medium (4 g asparagine, 0.5 g MgSO4, 0.5 g ferriammoniumcitrate, 0.5 g KH2PO4, 6 % glycerol per litre distilled water, pH adjusted to 7.4 with 10 % NH4OH) at 37 °C. At the beginning of the logarithmic phase the bacterial culture was harvested and resuspended in fresh medium to a cell density of approx. $10^8$ ml (0.4 to 0.5 extinction at 660 nm) and was incubated for 2 h. Following the addition of sufficient CaCl2 (0.001 M for Butyricum phage, and 0.004 M for Phlei phage) bacterial cells were infected with phage (multiplicity of infection: 2 to 5) and incubated overnight. The resulting phage lysate was centrifuged at 2000 rev/min for 20 min to remove bacterial cell debris. Large quantities of phages were grown in a fermentor. After phage infection the rate of aeration and of stirring was 1 l/min and 200 to 300 rev/min respectively.

Two types of purification procedure were employed: (i) ion exchange chromatography on triethyl-aminoethyl (TEAE) cellulose (Tikchonko, Koudelka & Borishpolez, 1963); (ii) a dextran sulphate 500-polyethylene glycol 6000 two phase system (Albertsson, 1967).

After treating with RNase (10 µg/ml) and DNase (10 µg/ml) the phage suspensions were further concentrated and purified by high speed centrifuging at 30000 g for 60 min and banding in a stepwise CsCl density gradient consisting of three layers constructed from the bottom of the tube as follows; 2 ml of 1.7 g/ml CsCl; 3 ml of 1.36 g/ml CsCl and 3 ml of 40 % (w/w) sucrose. Six to seven ml of phage suspension was layered on the gradient and centrifuged at 25000 rev/min for 2 h at 4 °C in the 4 × 20 ml swinging bucket rotor of the MSE high speed centrifuge. Biological assays of phage infectivity were carried out as described by Adams (1959).

For density determinations the concentrated phage suspension was adjusted with CsCl to a density of 1.4 g/ml and a pre-formed CsCl gradient was prepared in a 5 ml tube with CsCl solution of a density of 1.6 g/ml. This was centrifuged in an MSE Superspeed 50 ultracentri-
Table 1. Biophysical properties of phages Butyricum and Phlei

<table>
<thead>
<tr>
<th>Phage</th>
<th>Density (g/ml) in CsCl</th>
<th>$E_{260}$</th>
<th>$E_{260}$</th>
<th>$\delta_{260,v}$</th>
<th>% of the total phage weight</th>
<th>Phage head diameter in nm</th>
<th>Phage tail length in nm</th>
<th>Calculated mol. wt. $\times 10^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyricum</td>
<td>1.51</td>
<td>0.63</td>
<td>490</td>
<td>42</td>
<td>63.3</td>
<td>21.0</td>
<td>123</td>
<td>116</td>
</tr>
<tr>
<td>Phlei</td>
<td>1.51</td>
<td>0.63</td>
<td>410</td>
<td>34</td>
<td>63.4</td>
<td>15.8</td>
<td>116</td>
<td>123</td>
</tr>
</tbody>
</table>

* Calculations were carried out from the average results of two staining methods.
† The average diameter of the phage tail proved to be 5.5 nm.

Sedimentation constants of phage particles were measured with a Spinco Model E ultracentrifuge using either u.v. absorption or Schlieren optics. Phage sedimentation was performed at 11,200 rev/min in phage buffer at 20 °C using different concentrations of phages. The results were extrapolated to zero concentration to give values of $s_{0,w}$ for Butyricum and Phlei phages of 490S and 410S, respectively (Table 1).

The amount of DNA/phage particle was determined from the deoxyribose content as described by Hubbard, Matthew & Dubowik (1970). For analytical calculations one has to consider the relation of optical density of phage particles to weight. (One milligram of phage particles was found to be equivalent to 22.4 $E_{260}$ for Butyricum phages and 12.2 $E_{260}$ for Phlei phages). From the results of five experiments the average DNA content of Butyricum and Phlei was 42 and 34 %, respectively (Table 1).

Phage particles were examined in a JEOL 100B electron microscope and sizes were determined using two types of negative staining methods: (i) 0.5 % aqueous uranyl acetate, pH 4.3; (ii) 2% aqueous phosphotungstic acid, pH 6.3. Both procedures gave very similar dimensions for phages in question. The head dimensions of the two phages proved to be nearly identical, but a difference was seen in the tail length. Both phages have tails with distinct cross striations and at the distal end a knob can be clearly seen (Fig. 1). The shape of the phage head was determined by shadow casting technique as described by Nermut, Frank & Schäfer (1972). From the shadow of the phage particles we could establish that both phages have a head of icosahedral shape with threefold symmetry.

In this case the proportion between edge length and the icosahedron and the maximum diameter is 0.588 (Hosaka, 1965). The volume of the icosahedron can also be calculated by the following two equations

$$V = 2\cdot182L^3,$$

where $L$ indicates the edge length of the icosahedron

$$\frac{L}{D} = 0.588,$$

where $D$ is the maximum diameter of the phage head.
Fig. 1. Electron micrographs of purified Butyricum (a) and Phlei (b) phages negatively stained with uranyl acetate. Arrows point to knobs on the tail fibre terminus.
The volume of a phage head can be calculated by the above formulae; similarly, the volume of a phage tail can also be calculated using the dimensions of an equivalent cylinder.

From the phage DNA and protein content the density values for Phlei and Butyricum phages proved to be 1.51 and 1.54 g/ml respectively. Mol. wt. of Butyricum and Phlei phages, determined from the phage volume and density were calculated as $123 \times 10^6$ and $116 \times 10^6$, respectively. These dimensions and mol. wt. indicate that the two phages can be classified as large DNA bacteriophages.


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REFERENCES


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