Physical Properties of the DNA of a Temperate Providence Bacteriophage

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Coetzee, Smit & Prozesky (1966) described three serologically related phages PL25, PL26 and PL37 which produce generalized transduction in the Providence hosts. It was decided to investigate the physical properties of the nucleic acid of phage PL25 because this phage could usually be prepared in higher titre than its relatives.

Crude lysates (5 × 10^10 p.f.u./ml.) were partially purified by differential centrifugation. Pellets were resuspended in saline-citrate buffer (0.15 M-NaCl + 0.015 M-citrate) at pH 7.0 to a titre of about 5 × 10^12 p.f.u./ml. Phage DNA was prepared by the method of Mandell & Hershey (1960). The presence and concentration of DNA were determined by the diphenylamine reaction (Kupila, Bryan & Stern, 1960) and ultraviolet absorption at 260 nm. (1 µg DNA/ml. = 0.0205 absorbency units). Concentrations of DNA ranged from 400 to 600 µg./ml. and no protein was detected by the Lowry procedure (Lowry et al., 1951). Ultracentrifugation in a Spinco model E ultracentrifuge proved the DNA to be homogeneous. Base composition of the DNA was determined by means of the thermal denaturation temperature (Fig. 1) according to Doty & Marmur (1962). An average value of 87.0° was obtained which corresponds to a molar base composition of 43.2 % G+C.

Sedimentation coefficients were determined at concentrations between 10 and 50 µg. DNA/ml. according to the technique of Rosenblum & Schumaker (1963). Sedimentation coefficients depended on concentration as illustrated in Fig. 2. Extrapolation to zero concentration gave an S_20,w value of 32.5 × 10^{-13} sec. The molecular weight of the DNA was calculated with the use of various equations (Table I) and results varied from 21.1 to 27.9 × 10^6 with an average value of 24.4 × 10^6.

Electron microscopy was done according to Lang et al. (1967). Phage PL25 DNA was found to be a linear molecule and measurements of 30 molecules gave a contour length of 12.6 ± 0.6 µm. At an ionic strength of 0.20 it was assumed that the DNA was in the B configuration of the Watson-Crick model with a linear density of 196 daltons/Å (Thomas, 1966). From the contour length a molecular weight of 24.7 × 10^6 was calculated which is in agreement with the value obtained from sedimentation coefficient determinations. Chemical analysis of phage PL25 reveals that 48 % of the weight of the phage is accounted for by DNA and that the phage has a molecular weight of about 60 × 10^6 (Pitout, Conradie & Van Rensburg, 1969). From these findings the molecular weight of phage PL25 DNA is approximately 28 × 10^6. Using an average value of 662 molecular weight units/base pair (Schito, Rialdi & Pesce, 1966) the DNA of phage PL25 consists of about 3.7 × 10^4 nucleotide pairs. If the DNA retains the B crystalline form with an interbase separation of 3.46 Å (Caro, 1965) during the electron microscopic measurements, the over-all length of the polynucleotide chain would be 13.2 µm, which agrees with the contour length determined experimentally. Alkaline denaturation of the DNA (Studier, 1965) followed by renaturation yielded no circular molecules among more than 500 molecules studied. This suggests that phage PL25 DNA is a unique collection of DNA molecules (Thomas, 1966).
Penicillin spheroplasts were prepared (Lederberg, 1956) and transfection was performed by incubating spheroplasts at concentrations of about $5 \times 10^8$/ml for 15 min. in 1% Difco nutrient broth supplemented with 0.5 M-sucrose and 2% bovine serum albumin. Phage PL 25 DNA was then added to a final concentration of 50 $\mu$g./ml. and incubation continued. Controls were platings of DNA and spheroplasts individually on the bacterial host. At intervals duplicate 0.1 ml. samples were removed. One sample was plated directly on the indicator strain. The other was diluted 1/10 in water to rupture spheroplasts before plating. Free phage was first detected at 40 min. and titres increased rapidly to reach a plateau at 140 min. No plaques were ever seen on control plates. Maximal infection occurred at a spheroplast concentration of $5 \times 10^8$ to $1 \times 10^9$/ml. incubation mixture, and at low concentrations of DNA a linear relationship existed between the amount of DNA added and infectious units. DNA preparations stored at 4°C for one week lost all infectivity, which suggests that the molecules in solution are susceptible to degradation by low shear forces.

Results presented in Table 2 show that shearing forces and treatment by DNase abolish DNA infectivity, while pronase, trypsin and rabbit anti-PL 25 serum ($k$ value $= 84$ min.$^{-1}$) have no effect on infectivity. Efficiencies of phage DNA infection (Brody, Mackel & Evans, 1967) are not presented. The reason is that efficiencies varied

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**Table 1. Calculation of the molecular weight of bacteriophage PL 25 DNA with the use of different equations**

<table>
<thead>
<tr>
<th>Equation</th>
<th>Molecular weight $\times 10^8$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) $S_{50,w} = 0.063 M^{0.87}$</td>
<td>21.1</td>
<td>Doty, McGill &amp; Rice (1958)</td>
</tr>
<tr>
<td>(2) $S_{50,w} = 0.082 M^{0.246}$</td>
<td>25.6</td>
<td>Studier (1965)</td>
</tr>
<tr>
<td>(3) $S_{50,w} = 0.080 M^{0.85}$</td>
<td>27.9</td>
<td>Eigner &amp; Doty (1965)</td>
</tr>
<tr>
<td>(4) $0.445 \log M = 1.189 + \log(S_{50,w}^{0.8} - 2.7)$</td>
<td>24.7</td>
<td>Crothers &amp; Zimm (1965)</td>
</tr>
<tr>
<td>(5) $S_{50,w} = 7.20 \times 10^{-5} M^{6.805}$</td>
<td>22.3</td>
<td>Pitout (unpublished)</td>
</tr>
<tr>
<td>Average</td>
<td>24.3</td>
<td></td>
</tr>
</tbody>
</table>
markedly in different experiments depending on concentration of spheroplasts, age of the DNA and possible competence of the spheroplasts (Iliashenko, Dityatkin & Danileichenko, 1968).

Table 2. Summary of effects of various agents on the infectivity of PL25 DNA for NCTC 9211 spheroplasts

<table>
<thead>
<tr>
<th>Agent</th>
<th>Experimental procedure</th>
<th>Infectious units/ml</th>
</tr>
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<tbody>
<tr>
<td>Shear forces</td>
<td>DNA at a concentration of 25 μg./ml. passed 3 times through a 30-gauge needle.</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control: unsheared (25 μg./ml.)</td>
<td>3 x 10⁴</td>
</tr>
<tr>
<td>DNase</td>
<td>DNA (400 μg./ml.) incubated with 0.1 pancreatic DNase/ml. at 37° for 30 min. then diluted 50 times and assayed</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control: DNA incubated without enzyme</td>
<td>7 x 10⁴</td>
</tr>
<tr>
<td>Pronase</td>
<td>DNA (400 μg./ml.) incubated with 1.0 μg. pronase/ml. (pH 9.0) at 37° for 30 min. The mixture was diluted 50 times to safeguard spheroplasts from attack by pronase</td>
<td>2 x 10⁵</td>
</tr>
<tr>
<td></td>
<td>Control: DNA incubated without enzyme</td>
<td>2 x 10⁴</td>
</tr>
<tr>
<td>Trypsin</td>
<td>DNA (400 μg./ml.) incubated with 1.0 μg. trypsin/ml. (pH 7.0) at 37° for 30 min. The mixture was also diluted 50 times</td>
<td>2 x 10⁴</td>
</tr>
<tr>
<td></td>
<td>Control: DNA incubated without enzyme</td>
<td>2 x 10⁵</td>
</tr>
<tr>
<td>PL25-antiserum</td>
<td>DNA (400 μg./ml.) incubated with rabbit anti-PL25 serum for 30 min. After incubation the mixture was diluted 50-fold</td>
<td>4 x 10⁴</td>
</tr>
<tr>
<td></td>
<td>Control: no antiserum</td>
<td>4 x 10⁴</td>
</tr>
</tbody>
</table>

The ultracentrifugal behaviour of PL25 DNA is consistent with a monodisperse preparation and the size of the macromolecule in association with chemical data suggests that bacteriophage PL25 contains a single DNA molecule. The sedimentation coefficient, contour length and molecular weight compare well with the DNA of Salmonella transducing phage P22 (S₂₀,₅ = 31 to 36; length = 13.5 ± 0.5 μm.; M.W. = 26 x 10⁶), phage gh-1 of Pseudomonas putida (S₂₀,₅ = 31.0; M.W. = 23 x 10⁶) and phage T7 (S₂₀,₅ = 32.0 ± 0.8; length = 12.5 ± 0.6 μm.; M.W. = 25 x 10⁶) (Thomas & MacHattie, 1967).

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