Relationship Between the Sedimentation Coefficient and Molecular Weight of Bacteriophages

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SUMMARY

Bacteriophage PL25 was purified by centrifugation in caesium chloride. It has $S_{20,w} = 485$; $D_{20,w} = 0.68 \times 10^{-7} \text{ cm}^2/\text{sec}$, and $M.w. = 54.3 \times 10^4$. An equation was derived relating $S_{20,w}$ and molecular weight of bacteriophages.

INTRODUCTION

The molecular weight of bacteriophages can be derived from light-scattering (Schito, Rialdi & Pesce, 1966; Strauss & Sinsheimer, 1963), sedimentation-diffusion (Möller, 1964; Schito et al. 1966; Cummings & Kozloff, 1960; Davison & Freifelder, 1962; Swanby, 1959; Goldwasser & Putnam, 1952), sedimentation-viscosity (Schito et al. 1966) and sedimentation-equilibrium experiments (Dyson & van Holde, 1967), but determinations are cumbersome. Different equations relating $S_{20,w}$ and molecular weight of DNA were described by Josse & Eigner (1966). Since similar equations for bacteriophages would be useful we decided to investigate physical properties of phage PL25 with this in mind.

METHODS

Media. Difco brain-heart-infusion broth was used. Other media were described by Coetzee & Sacks (1960). Incubation temperature was 37°C.

Phages and hosts. Phage PL25 and its host strain Providence nctc 9211 (Coetzee, 1963) were used.

Preparation and purification of phage PL25. Phage lysates ($5 \times 10^{11}$ p.f.u./ml.) were prepared by an agar-layer method (Coetzee & Sacks, 1960). Phage stocks were sterilized by addition of 0.1 vol. chloroform. Crude lysates were purified by centrifugation at 8000 g for 30 min. at 10°C to remove agar. Supernatant fluids were then centrifuged at 30,000 g for 100 min. at 10°C, and the pellet suspended in 0.1 M-phosphate buffer, pH 6.8, or 0.15 M-NaCl + 0.015 M-citrate buffer, pH 7.0, to a titre of about $5 \times 10^{10}$ p.f.u./ml. Initially the phage was purified by subjecting it to charcoal (0.079 g. activated charcoal/ml. phage suspension), pronase (1 µg./ml. at pH 9.0) and combined charcoal + pronase treatments. These treatments were discarded on account of unsatis-
factory results. Therefore phage was finally purified by CsCl density gradient centrifugation. A phage suspension of 1·5 ml. was layered on to 3·5 ml. CsCl solution (12·2 g. CsCl in 14·4 ml. 0·1 M-phosphate buffer, pH 6·8) and centrifuged for 14 to 16 hr at 18,000 g in a Spinco SW 50 swinging bucket rotor. The phage band was removed with a syringe and again centrifuged. Ten-drop fractions were collected from the bottom of the tubes after puncture with a 22-gauge needle. The absorbance at 260 nm. of the fractions was measured in a Beckman DK-2A spectrophotometer using 1 mm. cells. Fractions with an absorption above 0·5 were collected and dialysed for 24 hr against five changes of 0·1 M-phosphate buffer, pH 6·8. The phage suspension was then assayed for infectivity.

Estimation of DNA content of phage PL25. This was determined according to the diphenylamine reaction (Kupila, Bryan & Stern, 1960).

Determination of sedimentation coefficient of phage PL25. Sedimentation coefficients of various concentrations of phage PL25 were determined in the Spinco model E ultracentrifuge equipped with ultraviolet optics. Relative concentrations of phage suspensions were expressed in absorbency units at 260 nm. and determinations were made between 0·2 and 1·5 units. The An-D and An-E rotors with 12 and 30 mm. cells were used. Phage suspensions were centrifuged at 20° and 9945 rev./min. Photographs were taken at 2 min. intervals on Kodak commercial film. Boundary positions were determined by scanning photographs with a Beckman Analytrol densitometer. All sedimentation coefficients were corrected to S 20,w and the limit sedimentation coefficient (S 0,w) was obtained by plotting S 20,w values against concentration of phage, followed by extrapolating to zero concentration.

Determination of diffusion coefficient of phage PL25. Boundary spreading in the ultracentrifuge cell was analysed in terms of a true diffusion coefficient (Möller, 1964). Diffusion coefficients were calculated from

\[ D = \frac{u^2(1 - S_{0w}t)}{4 y^2t}, \quad \frac{C_z}{C_0} = \frac{1}{2} \left[ 1 - \frac{2}{\sqrt{\pi}} \int_0^u e^{-\nu^2} d\nu \right]. \]

In these equations C z is the concentration at a distance z from the boundary; C 0 the initial concentration; u the mean distance in cm. at a time t from a level in the boundary where the concentration ratio (C/C 0) is 0·5 to the equidistant levels with concentration ratios (C/C 0) of 0·2 and 0·8 respectively. The factor y may be obtained from tables giving the numeral values for the well-known probability integral:

\[ \phi (y) = \frac{2}{\sqrt{\pi}} \int_0^y e^{-\nu^2} d\nu = 1 - \frac{2C}{C_0}, \]

for which values are given for definite values of C/C 0 (Svedberg & Pederson, 1940). Diffusion-coefficient experiments were performed at 5° using the An-E rotor with 12 and 30 mm. analytical cells. The rotor was spun at 9945 rev./min. for 3 min. to establish a permanent plateau. When the boundary had moved about 0·3 mm. from the meniscus, the rotor was decelerated to the preset low-speed value of 2095 rev./min. Diffusion coefficients were determined at the same concentrations as sedimentation coefficients. Exposure intervals were 64 min. All diffusion coefficients were corrected to D 20,w and plotted against phage concentration and extrapolated to zero concentration to give D 20,w.

Determination of partial specific volume. The density and partial specific volume (v) measurements were pycnometrically determined at 20·0° with the same solvent used
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in the sedimentation-diffusion measurements. The value was calculated according to Schachman (1957).

**Determination of molecular weight of phage PL25.** After standardization of the sedimentation and diffusion coefficients to the same solvent (water) and temperature (20°) (Svedberg & Pedersen, 1940), the molecular weight of phage PL25 was calculated from the Svedberg equation (Svedberg & Pedersen, 1940)

\[
M = \frac{RT_s}{D(1-\bar{v}\rho)}
\]

in which \( R = \) gas constant, \( 8.314 \times 10^7 \text{ erg/mol./degree} \); \( T = 293^\circ \text{K} \); \( s = S_{20,w}^\infty \) sec.; \( D = D_{20,w} \) cm.\(^2\)/sec.; \( \bar{v} = \) partial specific vol. cm.\(^3\)/g.; \( \rho = \) density of water at 293°K.

**RESULTS**

**Purification of phage PL25**

Only CsCl density gradient centrifugations yielded homogeneous preparations (Fig. 1, 2). It was necessary to repeat the CsCl centrifugation to remove all impurities. Purified phage sedimented as a single boundary indicative of homogeneous macro-
molecular material. In addition, the plateau region (solvent) did not contain any ultraviolet absorbing material (Fig. 2). The phage concentrations at various steps during purification are presented in Table 1.

Table 1. Purification of phage PL 25

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume (ml.)</th>
<th>P.f.u./ml.</th>
<th>Total p.f.u.</th>
<th>P.f.u. recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude lysate</td>
<td>200</td>
<td>3.0 x 10^{11}</td>
<td>6.0 x 10^{12}</td>
<td>100</td>
</tr>
<tr>
<td>Differential centrifugation</td>
<td>4.5</td>
<td>9.0 x 10^{12}</td>
<td>4.10 x 10^{13}</td>
<td>68</td>
</tr>
<tr>
<td>First CsCl density gradient</td>
<td>1.8</td>
<td>1.0 x 10^{13}</td>
<td>1.80 x 10^{13}</td>
<td>30</td>
</tr>
<tr>
<td>centrifugation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second CsCl density gradient</td>
<td>0.7</td>
<td>2.3 x 10^{13}</td>
<td>1.60 x 10^{13}</td>
<td>27</td>
</tr>
<tr>
<td>centrifugation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Physical properties of phage PL 25

Sedimentation coefficients of the phage were determined from velocity sedimentation runs at different concentrations. The sedimentation coefficient was independent of concentration and an average value of 485 was obtained for $S_{20,w}$. Schito et al. (1966) found the sedimentation coefficient of N4 coliphage to be concentration-dependent.

Fig. 3. A representative plot of $\bar{u}^2(1 - so^2t)$ against $t$ for the 20 (80%) C/C0 ratio in the boundary, where $\bar{u}$ denotes the mean distance in cm. at a time $t$ from a level in the boundary where the concentration ratio C/C0 is 0.5 to the equidistant levels with concentration ratios (C/C0) of 0.2 and 0.8 respectively. The slope of the line, $\tan \phi = \bar{u}^2(1 - so^2t)/t$, corresponding to the 20(80) point, was used for the calculation of the diffusion coefficient.

Spreading measurements made before, during, and after deceleration showed that, in the absence of external braking, no deterioration of the boundary took place during diffusion experiments. The diffusion coefficient was calculated by plotting $u^2(1 - so^2t)$ against time (Fig. 3), where $D = \bar{u}^2(1 - so^2t)/4y^2t$ and $y^2 = 1.417$ (Svedberg & Pedersen, 1940).
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The diffusion coefficient of phage PL25 was also independent of concentration and an average value of $0.68 \times 10^{-7}$ cm.$^2$/sec. was obtained. A value of $0.68$ ml./g. was calculated for the partial specific volume ($\bar{\rho}$), corresponding to a DNA content of 47% of the phage particle weight (Schito et al. 1966). The diphenylamine assay method yielded a value of 48%.

Table 2. Relationship between $S_{20,w}$ and molecular weight of bacteriophages

<table>
<thead>
<tr>
<th>Phage</th>
<th>$S_{20,w}$</th>
<th>Molecular weight $\times 10^6$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T6</td>
<td>1950</td>
<td>145.0</td>
<td>Goldwasser &amp; Putnam (1952)</td>
</tr>
<tr>
<td>T2</td>
<td>10666</td>
<td>220.0</td>
<td>Taylor, Epstein &amp; Lauffer (1955)</td>
</tr>
<tr>
<td>N4</td>
<td>615</td>
<td>83.0</td>
<td>Schito, Rialdi &amp; Pesce (1966)</td>
</tr>
<tr>
<td>T3</td>
<td>476</td>
<td>49.0</td>
<td>Swanby (1959)</td>
</tr>
<tr>
<td>MS2</td>
<td>81.5</td>
<td>5.3</td>
<td>Möller (1964)</td>
</tr>
<tr>
<td>φX-174</td>
<td>114.0</td>
<td>6.2</td>
<td>Sinsheimer (1959)</td>
</tr>
<tr>
<td>T7</td>
<td>487</td>
<td>38.0</td>
<td>Davison &amp; Freifelder (1962)</td>
</tr>
<tr>
<td>TP-84</td>
<td>436</td>
<td>50.0</td>
<td>Saunders &amp; Campbell (1966)</td>
</tr>
<tr>
<td>Lambda</td>
<td>416</td>
<td>57.0</td>
<td>Dyson &amp; Van Holde (1967)</td>
</tr>
<tr>
<td>PL25</td>
<td>485</td>
<td>54.0</td>
<td>This investigation</td>
</tr>
<tr>
<td>T2 (fast form)</td>
<td>1017</td>
<td>214.0</td>
<td>Cummings &amp; Kozloff (1960)</td>
</tr>
<tr>
<td>(slow form)</td>
<td>710</td>
<td>216.0</td>
<td></td>
</tr>
</tbody>
</table>

Determination of molecular weight of bacteriophages

The substitution of the various values obtained in the Svedberg equation yields a value of $54.3 \times 10^6$ for the molecular weight of phage PL25.

The molecular weight of phage PL25 was correlated with known molecular weight values of other bacteriophages (Table 2). A linear relationship was found between $S_{20,w}$ and the molecular weight of phage (on logarithmic scale). An empirical equation relating $S_{20,w}$ and molecular weight was derived:

$$S_{20,w} = 1.114 \times 10^{-3} \times M^{0.729}$$

DISCUSSION

The procedures of Van Holde (1960) and Mommaerts & Aldrich (1958) for determining the diffusion coefficient of phage PL25 gave unsatisfactory results. With the use of the Van Holde method (1960) the height/area value could not be determined accurately. No boundary could be obtained with the synthetic boundary cell in the Mommaerts & Aldrich (1958) procedure. This was probably due to sedimentation of phage before layering of buffer could take place. The method of Chervenka (1966) was attempted but was unsuccessful as sedimentation of the phage occurred at the lowest possible rev./min. setting of the centrifuge. The discrepancy in the diffusion coefficient with ultraviolet absorption optics (Fig. 3) was probably due to accumulation of phage at the bottom of the cell.

Results presented in Table 2 and Fig. 4 indicate that only one phage molecular weight deviates from the linear relationship with $S_{20,w}$. This value is for the slow form of phage T2 (Cummings & Kozloff, 1960). Electron micrographs obtained by different procedures show that the slow form has a longer head than the fast form. This abnormality is probably the cause of the anomalous diffusion coefficient and molecular weight of the slow form of T2. This form is obtained at a pH value of 5.7, while sedimentation coefficients are normally measured at about neutral pH.
An empirical equation for the determination of molecular weights of spherical RNA phages has been derived by Marvin & Hoffmann-Berling (1963). In this equation

\[ M = 1150 \frac{S_{20,w} \times \bar{v}}{1 - \bar{v}} \]

where \( S_{20,w} \) is the limit sedimentation coefficient; \( \bar{v} \) the partial specific volume and \( \bar{v} \) the density of the solution. Substituting the values of 485 and 0.68 for \( S_{20,w} \) and \( \bar{v} \) respectively in the Marvin & Hoffmann-Berling equation, an \( M \) value of \( 56.0 \times 10^6 \) is obtained for phage PL25, which is in close agreement with the value procured from our equation.

![Sedimentation coefficient as a function of molecular weight](image)

Fig. 4. Sedimentation coefficient as a function of molecular weight. Bacteriophage MS2, \( \phi X-174 \), T7, T3, TP-84, lambda, PL25, N4, T6 and T2 are represented by O, O, D, III, A, T, V, ~, and G, respectively. References to these phages are listed in Table 2.

The physical characterization of macromolecules assists in clarifying their biological roles. This might also apply to bacteriophages. Our results indicate that molecular weights of phages can be determined from a knowledge of their sedimentation coefficients.

REFERENCES


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