A Fibrous DNA Phage (fd) and a Spherical RNA Phage (fr) Specific for Male Strains of E coli

Part II. Physical Characteristics

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The phage fd is a flexible rod-shaped virus with a sedimentation constant of 40 s. It contains single-stranded DNA instead of RNA. Bacteriophage fr is a spherical RNA-containing virus, similar to f2 and other RNA bacteriophages. The preparation and chemical characteristics of these two bacteriophages are described in the preceding paper (Part I).

2. Materials and Methods

(a) Preparation

The growth and purification of the virus particles and the preparation of high molecular weight nucleic acid are described in Part I. Alkaline degradation was as follows. To 1 ml iced fr phage in dilute buffer at a concentration between 1 and 20 mg/ml was added 0.1 M NaOH to bring to pH 11.

Concentration measurements

Concentrations were determined by measuring UV absorption at 260 μm on suitable dilutions and then using the specific absorption coefficients given in Part I.

(c) Buffers

Measurements were made in 0.1 M NaCl containing 0.02 M tris adjusted to pH 7.3 with HCl, unless otherwise stated. Phosphate buffer is 0.1 M Na2HPO4 and 0.1 M NaH2PO4 mixed in appropriate ratio to give the required pH, and then diluted to the stated concentration. Citrate buffer is 1 M Na citrate adjusted to pH 7.3 with NaOH and then diluted. Borate buffer is 0.05 M Na borate, adjusted to the required pH with 0.2 M boric acid or 0.2 M NaOH.

(d) Sedimentation

Sedimentation constants were measured with a Spinco model E ultracentrifuge, using either ultraviolet


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absorption optics or schlieren optics. The cell had a light path length of 12 mm. Ultraviolet absorption pictures were densitometered with a Joyce-Loebl Mk III B microdensitometer. Schlieren pictures were measured with a travelling microscope. Sedimentation constants were determined by plotting log (distance to axis of rotation) versus time. Measurements were made at 20.0 ± 0.1 °C unless otherwise stated. The sedimentation constants were corrected for viscosity of the solvent, and for temperature where necessary.

(e) Viscosity

Viscosities of fd phage were measured at various rates of shear, using Ostwald viscometers for the higher rates of shear and a viscometer consisting of a coil of 1 mm diameter capillary, 1.5 m long, for low rates of shear (we are grateful to Dr. Wagner for the use of this viscometer). The average rate of shear was calculated for solvent for each viscometer by the equation

\[ G = \frac{4V}{\pi R^2 t_0} \]

Viscosities of fr and fr-RNA were measured in an Ostwald viscometer having an average rate of shear of 1200 sec^{-1}, and was not extrapolated to zero rate of shear. Temperature was 20.0 ± 0.1 °C unless otherwise stated. Specific viscosity is given by

\[ \eta_{sp} = \frac{\eta - \eta_0}{\eta_0} t_0 \]

For dilute solutions one can set \( \eta = \eta_0 \) but for more concentrated solutions \( \eta \) is appreciably larger than \( \eta_0 \). The value of \( \eta \) can be calculated from

\[ \eta = \eta_0 + c(1 - \eta_0) \]

where \( c \) is the concentration in g/cc. This correction was applied in calculating the viscosity of fr solutions, and ignored for other viscosity measurements.

(f) Diffusion

The capillary cell of the Spinco E ultracentrifuge was used for diffusion experiments. At a rotor speed of 9341 rpm, no rotor vibration was detectable with the AN-D rotor. Determination of the diffusion coefficient in the ultracentrifuge has the advantage that a very sharp initial boundary is obtained with no difficulty; it has the disadvantage that the fr phage sediments within about 3 hours at the rotor speeds needed for stability. In calculating results, boundary sharpening due to sedimentation was ignored. Either schlieren or interference optics were used and patterns were evaluated according to Gosting. Measurements were made at 20.0 ± 0.1 °C.

(g) Partial specific volume

A 5 cc pycnometer was used to measure the partial specific volume of phage in solution. The solutions had concentrations of 10–20 mg/ml. Phage concentrations were determined from UV absorption on dilution series.

(h) Electrophoresis

Membranfolien (Membranfilter, Göttingen) were used as carrier material for electrophoresis. An LKB Paper electrophoresis apparatus (LKB-Produkter, Stockholm) was used. The membranfolien were stained with amido black and washed as recommended by the manufacturers.

(i) Electron microscopy

For negative staining, a modification of the procedure of Brenner and Horne was used. Phage at a concentration of about 10^{13}/ml were diluted directly into 2% neutral phosphotungstate. A drop of this solution was placed on a formvar coated grid and allowed to dry.

Oriented fd phage were prepared by placing a drop of phage dissolved in water on a grid and allowing it to dry; then a drop of water was placed on the grid and drawn off from one side with a piece of filter paper. This washing was repeated 5–10 times. Then the grids were shadowed perpendicular to the direction of flow.

Electron micrographs were made with a Siemens Elmiskop I. The instrumental magnification was calibrated using polystyrene latex spheres of 2600 Å diameter, supplied to this laboratory some years ago by Dr. R. C. Williams. The magnification thus calculated was 5% smaller than the magnification given by the manufacturers (e.g. instead of 8000 x, true magnification is 7600 x). Distances were measured directly from the electron micrograph plate, using a travelling microscope.

(j) Calculation

The following symbols are used: \( S_{20\times} \) is sedimentation constant in Svedbergs corrected to 20 °C, water and zero concentration; \( s \) is sedimentation constant in seconds; \( D_{20\times} \) is translational diffusion constant in cm^2 sec^{-1} corrected to 20 °C, water and zero concentration; \( \Theta \) is rotary diffusion constant in sec^{-1}; \( V \) is volume (cc) and \( R \) is capillary radius (cm) for a viscometer; \( G \) is viscosity rate of shear (sec^{-1}); \( t_0 \) is flow time for solvent and \( t \) is flow time for solution in a viscometer (sec); \( \eta_0 \) is measured intrinsic viscosity in dl/g; \( v \) is form viscosity increment (2.5 for a sphere) and \( \nu \) is measured viscosity increment in dimensionless units; \( \nu_0 \) is viscosity of solvent (taken as 0.01 poise at 20 °C in calculations); \( a/b \) is axial ratio of equivalent ellipsoid; \( f \) is measured frictional coefficient and \( f_0 \) is frictional coefficient for anhydrous sphere of the same molecular weight (gm sec^{-1}); \( \eta_0 \) is density of solvent (taken as 1 g/cc in calculations), \( \eta \) is density of solution (taken as 1 g/cc unless otherwise stated); \( w \) is gram water of hydration per gram virus; \( \bar{v} \) is partial specific volume of solute in cc/g; \( c \) is concentration (in g/dl.

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6 J. T. Yang, Advances Protein Chem. 16, 323 [1961].
7 L. J. Gosting, Advances Protein Chem. 11, 429 [1956].
3. Results and Discussion

3.1. fd Phage

(a) Partial specific volume

The measured partial specific volume is 0.71 cc/g ± 2 percent.

(b) Sedimentation

The concentration dependence of the fd sedimentation constant is shown in Fig. 1. The $S_{20,w}^0$ and the coefficient $K$ in the equation

$$1 \frac{S_{20,w}^0}{S_{20,w}^0} = 1 + Kc$$

for fd in various NaCl concentrations are listed in Table 1. No significant salt effect is apparent over a ten-fold range of salt concentration. An average $S_{20,w}^0 = 40 s ± 2\%$ was used for all calculations.

![Fig. 1. Concentration dependence of fd sedimentation constant in 0.2 M NaCl, 0.001 M tris pH 7.3.](image)

The sedimentation constant of a rod-shaped molecule is relatively insensitive to the axial ratio $a/b$ and therefore is a measure of its diameter $D$ or mass per unit length $\frac{M}{2a}$.

From the equation

$$\frac{M}{2a} = \frac{3\pi \eta_0}{(1-\frac{v}{2})} \frac{s N}{\ln \left(\frac{2a}{b}\right)} m. w. cm^{-1}$$

one calculates a mass per length of 1430 m.w./Å, with $a/b = 125$ (see below).

The mass per length $\frac{M}{2a}$ combined with the partial specific volume gives an average diameter for the phage (assuming circular cross-section and no hydration):

$$D = 2 \left(\frac{M \bar{v} \times 10^{24}}{2a N \pi}\right)^{1/2} Å$$

The diameter calculated is 46 Å.

(c) Viscosity

Concentration dependence of viscosity at a shear rate of 1200 sec$^{-1}$ is shown in Fig. 2. Intrinsic viscosity $[\eta]$ and the constant $K'$ in the Huggins equation

$$\frac{\eta_{sp}}{c} = [\eta] + K'[\eta]^2 c$$

are listed for fd in various NaCl concentrations in Table 1. No dependence of $[\eta]$ on salt concentration was observed. A mean value $[\eta] = 4.7 dl/g ± 5\%$ was used.

![Fig. 2. Concentration dependence of fd viscosity in 0.2 M NaCl, 0.001 M tris pH 7.3.](image)

Table 1. Concentration dependence of sedimentation constant and intrinsic viscosity at 1200 sec$^{-1}$ rate of shear for fd phage in various NaCl concentrations. All solutions contained 0.001 M tris pH 7.3.

<table>
<thead>
<tr>
<th>Molarity of NaCl</th>
<th>$S_{20,w}^0$ [Svedberg]</th>
<th>$K$ [dl/g]</th>
<th>$[\eta]$ [dl/g]</th>
<th>$K'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>40.6</td>
<td>1.0</td>
<td>4.6</td>
<td>3.3</td>
</tr>
<tr>
<td>0.05</td>
<td>41.1</td>
<td>1.3</td>
<td>4.8</td>
<td>2.6</td>
</tr>
<tr>
<td>0.1</td>
<td>40.0</td>
<td>0.6</td>
<td>4.9</td>
<td>1.3</td>
</tr>
<tr>
<td>0.2</td>
<td>39.4</td>
<td>0.2</td>
<td>4.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>


10 M. L. Huggins, J. Amer. chem. Soc. 64, 2716 [1942].
Intrinsic viscosity was extrapolated to zero rate of shear using a series of different viscometers (Table 2). The value for zero rate of shear is 8.5 dl/g ± 10 percent.

<table>
<thead>
<tr>
<th>$G$ [sec$^{-1}$]</th>
<th>[η]$_G$ [dl/gm]</th>
<th>[η]$_{G=0}$</th>
<th>$\Theta$ [sec$^{-1}$]</th>
<th>$2a$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.5</td>
<td>75</td>
<td>6400 (extrap. val.)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>8.2</td>
<td>0.965</td>
<td>71</td>
<td>6400</td>
</tr>
<tr>
<td>600</td>
<td>6.0</td>
<td>0.706</td>
<td>100</td>
<td>5800</td>
</tr>
<tr>
<td>1200</td>
<td>4.7</td>
<td>0.553</td>
<td>114</td>
<td>5500</td>
</tr>
<tr>
<td>1800</td>
<td>1.5</td>
<td>0.177</td>
<td>133</td>
<td>5200</td>
</tr>
</tbody>
</table>

Table 2. Non-Newtonian viscosity and rotary diffusion constant of fd.

The measured viscosity increment

$$\nu' = \frac{100[\eta]}{v}$$

is related to the form viscosity increment $\nu$ and the hydration $w$ by

$$\nu' = 1 + w \frac{v}{\Theta}.$$  

The measured viscosity increment for fd is 1200. Assuming hydration of 0.25 g water per gram virus, $\nu$ is 885 and the axial ratio is 125.$^{11}$

The dependence of viscosity on rate of shear $G$ can be used to calculate a value for the rotary diffusion constant $\Theta$. For a rod-shaped particle, high rates of shear tend to orient the particle parallel to the direction of flow, so that the measured intrinsic viscosity [η]$_G$ is less than the viscosity extrapolated to zero rate of shear, [η]$_{G=0}$. This tendency toward orientation is opposed by the rotary diffusion of the particle. The relation between these two effects has been expressed quantitatively as a table of the ratio $G/\Theta$ versus the ratio [η]$_G$/[η]$_{G=0}$. Thus $\Theta$ can be found by measuring [η]$_G$/[η]$_{G=0}$ at a given rate of shear.

Since $\Theta$ for a rod-shaped particle is relatively insensitive to the diameter of the particle, $\Theta$ can be used to calculate the length $2a$ of the particle.$^{11}$:

$$a = \left( \frac{3KT}{16\pi\eta_b\Theta(2\ln2\frac{a}{b} - 1)\eta_0} \right)^{\frac{1}{2}} \text{cm}.$$  

Table 2 shows the values of $\Theta$ and corresponding values of $2a$ calculated for various rates of shear $G$.

For $G$ extrapolated to zero, $\Theta = 75$ sec$^{-1}$ (Fig. 3). The dependence of $\Theta$ on $G$ probably indicates inhomogeneity in the sample. The calculated length $2a = 6400$ Å is subject to considerable error in the extrapolation to zero rate of shear: it is probably accurate to no more than ± 30 percent.

![Fig. 3. Dependence of calculated rotary diffusion constant $\Theta$ on rate of shear $G$ for fd phage.](image)

(d) **Electron microscopy**

For measurement of particle length, the phage were diluted in water to a concentration of $10^{12}$ to $10^{13}$ per ml and oriented by flow on electron microscope grids (Fig. 4). A total of 207 particles from two separate lysates were measured. Only those particles which were clearly not side-by-side aggregates were measured. The results are presented in Fig. 5. About 85% of the particles had lengths of 7600 ± 500 Å. Fig. 4 illustrates two characteristic tendencies of the particles: aggregation in parallel groups and aggregation of two particles end-to-end.

![Fig. 5. Length distribution of fd phage as measured by electron microscopy.](image)


$^{12}$ J. T. Yang, J. Amer. chem. Soc. 80, 1783 [1958].
A maximum diameter for the phage particles was measured on aggregated phage, where 5—10 phage lay parallel over short regions (Fig. 4*). The width of the aggregate divided by the number of molecules in the group gives an average center-to-center distance of \( D = 50 \pm 10 \text{Å} \). The axial ratio of the equivalent ellipsoid with the same length and volume is given by

\[
a : b = \left( \frac{2}{3} \right) L / D
\]

so \( a/b = 125 \).

(c) **Molecular weight**

Probably the best value for the molecular weight can be calculated using the mass per length from sedimentation and the length from electron microscopy. The value obtained is \( 10.9 \times 10^6 \text{ m.w.} \).

The molecular weight can also be calculated from sedimentation and viscosity measurements\(^{13}\). If the axial ratio is 125 the molecular weight is \( 11.8 \times 10^6 \).

The electron microscope measurements are more accurate, but measure the dried molecule; viscosity measurements are subject to more error but are made under conditions similar to the sedimentation measurements. The molecular weight \( 11.3 \times 10^6 \) is probably accurate to \( \pm 10 \% \).

The picture of the molecule obtained from various measurements is presented in Table 3.

<table>
<thead>
<tr>
<th>Observed</th>
<th>mass length [ mw/Å ]</th>
<th>calculated length [ Å ]</th>
<th>Diameter [ Å ]</th>
<th>axial ratio of equivalent ellipsoid</th>
<th>molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>S EM ([\eta] )</td>
<td>1430</td>
<td>7600</td>
<td>46</td>
<td>50</td>
<td>125</td>
</tr>
<tr>
<td>S + EM</td>
<td>6400</td>
<td>125</td>
<td>125</td>
<td>11.8</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Calculated physical properties of fd and observed quantities from which they were calculated. Partial specific volume (not listed) is involved in all calculations except those dependent solely on EM measurement.

3.2. **fd DNA**

(a) **Sedimentation**

The sedimentation behavior of fd DNA is quite similar to that of \( \Phi X 174 \) DNA\(^{14}\). In 0.2 M NaCl, 0.001 M phosphate \( p_H 7.5 \) the DNA sediments with 24.4 s, whereas \( \Phi X 174 \) DNA sediments with 23.8 s in the same buffer. In 0.02 M citrate two ultracentrifuge bands appear, of 13.4 s and 14.7 s (Fig. 6); \( \Phi X 174 \) DNA shows a similar effect in dilute buffer.

(b) **Molecular weight**

Sedimentation data alone suggests that the molecular weight of fd DNA is similar to that from \( \Phi X 174 \)\(^{14}\): \( 1.6 - 1.8 \times 10^6 \). This is somewhat higher than the value \( 1.4 \times 10^6 \) obtained from the molecular weight and DNA content of the whole phage.

The presence of two ultracentrifuge bands at low ionic strength has been shown for \( \Phi X 174 \) DNA to be due to the existence of a closed ring configuration for the DNA\(^{15}\). If the same effect for fd DNA is due to the same cause, the packing of DNA in the phage particle would seem to involve a single DNA strand running from one end of the particle to the other and then back again.

3.3. **Light fd particle**

(a) **Sedimentation**

The light fd particle sediments with \( S_{20,w}^0 = 36.5 \text{ s} \). Assuming a partial specific volume of 0.72 cc/g, the mass per length from equation (2) is 1310 mw/Å.

3.4. **fr phage**

(a) **Partial specific volume**

The measured partial specific volume is 0.69 cc/g \( \pm 2 \% \).

\[ \text{Fig. 7. Concentration dependence of fr sedimentation constant in 0.1 M NaCl, 0.02 tris pH 7.3.} \]

\( ^{13} \) H. A. Scheraga a. L. Mandelkern, J. Amer. chem. Soc. 75, 179 [1953].

\( ^{14} \) R. L. Sinsheimer, J. molecular Biol. 1, 43 [1959].

\( ^{15} \) W. Fiers a. R. L. Sinsheimer, J. molecular Biol. 5, 424 [1962].

* Figure 4, 6 and 10 see table p. 888 a and b.
Fig. 4. Top. Electron micrograph of fd oriented by flow and shadowed perpendicular to the direction of flow. Bottom. Electron micrograph of fd stained with PTA.

Zeitschrift für Naturforschung 18 b, p. 888 a.
Fig. 6. Sedimentation pattern of fd DNA in 0.02 M citrate. Pictures taken every 4 minutes at a rotor speed of 59,780 rpm.

Fig. 10. Left. Electron micrograph of shadowed preparation of fr. Right. Electron micrograph of PTA stained preparation of fr.

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(b) Sedimentation

The concentration dependence of fr sedimentation is illustrated in Fig. 7. Equation 1 fits the data with $S_{20,w}^0 = 79.0$ and $K = 0.1 \text{ dl/g}$. The sedimentation constant does not vary noticeably with change in buffer (Table 4). An average value $S_{20,w}^0 = 79 \pm 2\%$ was used for all calculations.

<table>
<thead>
<tr>
<th>Salt [M]</th>
<th>pH</th>
<th>$S_{20,w}^0$ [Svedbergs]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 NaCl</td>
<td>7.3</td>
<td>79.9</td>
</tr>
<tr>
<td>0.1 NaCl</td>
<td>7.3</td>
<td>79.0</td>
</tr>
<tr>
<td>0.1 NaCl</td>
<td>8.5</td>
<td>79.1</td>
</tr>
<tr>
<td>0.1 NaCl</td>
<td>7.3</td>
<td>78.6</td>
</tr>
<tr>
<td>0.3 NaCl</td>
<td>7.3</td>
<td>75.1</td>
</tr>
<tr>
<td>0.0015 MgAc</td>
<td>7.3</td>
<td>79.5</td>
</tr>
<tr>
<td>0.05 KCl</td>
<td>7.3</td>
<td>77.5</td>
</tr>
<tr>
<td>0.3 KCl</td>
<td>7.3</td>
<td>77.5</td>
</tr>
</tbody>
</table>

Table 4. Sedimentation behavior of fr under various salt and pH conditions. Measurements were made with UV optics at a concentration of 0.01 g/dl. All solutions contained 0.02 M tris.

(c) Viscosity

Reduced specific viscosity is plotted against concentration in Fig. 8. The intrinsic viscosity $[\eta]$ is 0.044 dl/g. Since high concentrations were necessary to give a measurable viscosity, the extrapolation to zero concentration is subject to error: $[\eta]$ is probably accurate to no more than $\pm 5\%$. The constant $K'$ in the Huggins equation (3) is 1.2. This compares with the theoretical value 2.0 for unhydrated spheres 16.

(e) Electron Microscopy

The particle diameter measured on electron micrographs is 210 Å $\pm 10\%$. The nature of the virus fine structure is not clear (Fig. 10).

(f) Isoelectric point

Electrophoresis on membranofilen in borate buffer shows that the isoelectric point of fr is about pH 9 ($\pm 10\%$).

(g) Alkaline degradation

Treatment at pH 11 for ten minutes results in degradation of the phage to three ultracentrifuge components with $S$ of about 60 s, 40 s and 4 s ($\pm 10\%$). A qualitatively similar effect has been noted for bromegrass mosaic virus 17. The area under the 60 s schlieren peak is about twice that under the 40 s peak; the 4 s peak varies in size but is of the same order of magnitude as the other two peaks. Measurement of the absorption ratio at 260 and 280 mμ for fractions taken from a sucrose gradient 18 suggests that the 60 s and 40 s fractions are nucleoprotein, whereas the 4 s fraction is nearly pure RNA.

(h) Molecular weight

A rapid and quite accurate method (see appendix) of determining molecular weight of a small spherical

virus requires only the sedimentation constant and the partial specific volume:

\[ M = 1150 \left( \frac{S}{1 - \bar{v}} \right)^{\frac{1}{2}} \bar{v}^{\frac{1}{2}} \]

The value for fr phage is \(3.9 \times 10^6\) m.w.

If one assumes that the virus particle is a perfect sphere, its molecular weight can be calculated using sedimentation and viscosity\(^\text{13}\):

\[ M = \left( \frac{N s \bar{v}_0}{2.12 \times 10^6 (1 - \bar{v} \rho_0)} \right)^{\frac{1}{2}} \eta^{\frac{1}{2}}. \]

The value for fr phage is \(4.1 \times 10^6\).

From the equation\(^\text{19}\)

\[ M = \frac{R T s}{D(1 - \bar{v} \rho_0)} \]

one obtains \(4.3 \times 10^6\) for the molecular weight of fr phage.

The mean molecular weight \(4.1 \times 10^6\) is probably accurate to \(\pm 10\) percent. The radius of the dehydrated phage particle

\[ r = \left( \frac{3 M \bar{v}}{4 \pi N} \right)^{\frac{1}{2}} \text{cm} \]

is 104 Å.

(i) **Frictional ratio and solvation**

The frictional ratio of a spherical particle in terms of the viscosity is

\[ f = \left( \frac{100 \eta}{2.5 \bar{v}} \right)^{\frac{1}{2}}. \]

The value for fr phage is 1.37.

The frictional ratio of a spherical particle in terms of sedimentation and diffusion is

\[ f = \frac{1}{\eta_0} \left( \frac{k T}{6 \pi D} \left( \frac{1}{9 s \bar{v}} \right)^{\frac{1}{2}} \right)^{\frac{1}{2}} \]

The value calculated for fr is 1.41.

If the phage is a perfect sphere with a frictional ratio of 1.39, it contains 1.2 g water per gram phage. This is a maximum value for hydration, since the phage is in fact polyhedral. If this water were all present as a shell surrounding the phage, the shell would be about 40 Å thick. It is more likely that most of the water is contained within the phage particle.

3.5. fr RNA

(a) **Sedimentation**

\(S_{20,w}^0\) for fr-RNA measured in 0.02 M citrate at 4 °C is 25 s (Fig. 11).

Sedimentation constants \(S_{20,w}^0\) measured with UV absorption optics are listed for various salt and temperature conditions in Table 5. The sedimentation constants are consistently 10 - 15% higher when measured at 4 °C and corrected to 20 °C than when measured at 20 °C. The effect is illustrated by Fig. 12. This change is reversible and is therefore probably due not to depolymerization at higher temperatures but to a contraction of the coiled molecules at low temperatures.

![Fig. 11. Concentration dependence of fr RNA sedimentation constant in 0.02 M citrate at 4 °C.](image)

![Fig. 12. Temperature dependence of fr RNA sedimentation constant in 0.02 M phosphate.](image)

The sedimentation constant in 0.1 M NaCl is found to be considerably larger than in 0.02 M phosphate (Table 5). This effect is reversible. Such an increase in \(S_{20,w}^0\) could be due to a contraction of the coiled molecule or to a specific aggregation of two RNA strands. This sedimentation effect is especially noteworthy in view of the fact that UV absorption of fr RNA changes scarcely at all in the range 0.1 M to 0.01 M NaCl (Part I). This is in...
Table 5. Sedimentation constants of fr RNA under various conditions. Measurements were made with UV optics at a concentration of about 0.004 g/dl. p[H] was 7.0—7.5.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Temperature [°C]</th>
<th>$S_{20, w}$ [Svedbergs]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02 phosphate</td>
<td>4</td>
<td>23.6</td>
</tr>
<tr>
<td>0.02 phosphate</td>
<td>20</td>
<td>21.2</td>
</tr>
<tr>
<td>0.02 citrate</td>
<td>4</td>
<td>24.6</td>
</tr>
<tr>
<td>0.02 citrate</td>
<td>20</td>
<td>20.9</td>
</tr>
<tr>
<td>0.1 NaCl, 0.02 tris</td>
<td>20</td>
<td>27.4</td>
</tr>
</tbody>
</table>

Fig. 13. Concentration dependence of fr RNA viscosity, measured at 4 °C in 0.02 M citrate.

contrast to the correlation between sedimentation and absorption behavior found for TMV RNA. The salt effect for fr RNA is most dramatically demonstrated in an experiment performed by W. Vielmetter (private communication): radioactive fr RNA was mixed with larger amounts of 23 s ribosomal RNA in 0.1 M NaCl, 0.01 M NaAcetate, p[H] 5.3 and analyzed by sucrose gradient centrifugation. The radioactivity appeared in a band ahead of the 23 s band, at about 28 s. Thus under identical conditions fr RNA sediments considerably faster than ribosomal RNA of similar molecular weight.

(b) Viscosity

The concentration dependence of viscosity in 0.02 M citrate at 4 °C is shown in Fig. 12. The intrinsic viscosity $[\eta]$ is 0.83 dl/g; the Huggins constant $K'$ in Eq. 3 is 0.78.

(c) Molecular weight

Molecular weight was calculated from the Scheraga-Mandelkern equation using $S_{20, w}^0$ and $[\eta]$, as measured at 4 °C in 0.02 M citrate, $\beta$ as determined for TMV RNA at 6 °C, and $v = 0.55$ cc/g. The value calculated is $1.3 \times 10^6$, in satisfactory agreement with the value $1.2 \times 10^6$ calculated from the molecular weight and 30% RNA content (Part I) of the phage particle.

3.6. Light fr particle

(a) Sedimentation and molecular weight

The light fr particle sediments with $S_{20, w}^0 = 71 s$. Assuming no change in form or partial specific volume, molecular weight of a sphere changes as $s^{3/2}$, so the light particle molecular weight would be $3.5 \times 10^6$. This is a minimum molecular weight, for the light particle is probably less compact than the fr phage.

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Appendix

Molecular Weight of a Spherical Virus in Terms of the Sedimentation Constant and Nucleic Acid Content

The molecular weight of a sphere can be expressed in terms of the sedimentation constant and the viscosity increment as

$$M = 6\pi N \left( \frac{\eta_0 s}{1 - \frac{v}{2}} \right)^{3/2} \left( \frac{9 \beta v'}{2} \right)^{1/2}.$$

Combining constants and expressing $s$ as Svedbergs gives

$$M = 484 \left( \frac{S_{20, w}^0 v/v'}{1 - \frac{v}{2}} \right)^{3/2} (\nu')^{1/2}.$$ (1)

For small spherical viruses (molecular weight $1 - 10 \times 10^6$) it is probably reasonable to assume that the viscosity increment will be roughly the same for different viruses. Thus equation (1) enables one to determine a rough value of the molecular weight of a spherical virus using only the sedimentation constant and the partial specific volume.

1 H. Boedtner, J. molecular Biol. 2, 171 [1960].
21 R. Haselkorn, J. molecular Biol. 4, 357 [1962].
Table 1. Physical and chemical data for small spherical viruses. Viruses are RNA-containing plant viruses unless otherwise noted. In general data were taken from the most recent work when there was a choice. Calculated \( M \) is that given by Eq. 4. a Data for these viruses were not included in Figs. 1 and 2. b Contains single-stranded DNA. c Animal viruses. d Bacterial viruses.

In order to determine \( \nu' \) in equation (1), \( M \) versus \( S_{20,\text{w}}^{0.8 \frac{\nu'}{1-\nu'}} \) was plotted for various viruses (Table 1) on a double log scale, and the best line with slope \( \frac{3}{2} \) was drawn through the points (Fig. 1). The equation derived from this line is

\[
M = 1150 \left( \frac{S_{20,\text{w}}^{0.8 \frac{\nu'}{1-\nu'}}}{1-\nu'} \right)^{\frac{3}{2}} \tag{2}
\]

Comparison of equation (1) and (2) gives 5.6 for \( \nu' \).

For a perfect sphere, \( \nu' = 2.5 \left( 1 + \frac{w}{\varphi w} \right) \), where \( w \) is gram water per gram virus. If \( \varphi = 0.70 \text{cc/g} \), one calculates 0.8 gram water per gram virus. This is a maximum value, since in practice \( \nu' \) includes a slight asymmetry factor.
An approximate value for the partial specific volume can be obtained from the nucleic acid content if one assumes values $\bar{v}$ and $\bar{v}_p$ for the nucleic acid and protein:

$$\bar{v} = n \bar{v}_n + (1-n)\bar{v}_p$$

where $n$ is the weight fraction nucleic acid. In Fig. 2, nucleic acid content is plotted against $\bar{v}$ for the data of Table 1. The line which best fits the points gives $\bar{v}_n = 0.53 \text{ cc/g}$, $\bar{v}_p = 0.75 \text{ cc/g}$. For $\bar{v}$ between 0.68 and 0.72, the empirical equation

$$\frac{\bar{v}^{1/3}}{1-\bar{v}} = 3.55 - 2.5 n \quad (3)$$

fits the data to within 1 percent. Combining equations (2) and (3) gives

$$M = 1150 (S^{0.5}_0, (3.55 - 2.5 n)^{1/2} \quad (4)$$

for the molecular weight of a spherical virus in terms of the sedimentation constant and the nucleic acid content.

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Ein fädiger DNS-Phage (fd) und ein sphärischer RNS-Phage (fr) wirtsspezifisch für männliche Stämme von E. coli

III. Biologisches Verhalten von fd und fr

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fd and fr adsorb to male strains of E. coli and infect female cells, after the fd-DNA (or the fr-RNA $^{15}$) have been deproteinized by phenol and after the cells have been converted to the form of spheroplasts.

fd is very heat resistant, highly antigenic and poorly adsorbing. The latency period of intracellular multiplication is 10 min (in Tryptone broth at 37 °C). The most unusual property seems to be that fd is the only phage on record which is liberated by the host cell without destruction of the host cell. The evidence for this is threefold: 1. In infected cultures phage is liberated at the rate of about 300 phage particles per bacterium per cell generation, and the growth rate of these cultures is indistinguishable from that of controls. 2. In such cultures no significant amounts of bacterial enzyme are liberated. 3. In single burst experiments it appears that more than 60% of the individual cells have liberated around 450 phage particles after about 20 min. and later produce bacterial growth as shown by turbidity. Non-lytic infection gives rise to an unstable carrier state. The phage is lost from the cells if superinfection is prevented by the addition of fd-antiserum. Lytic mutants of fd have been recorded.

fr in its properties is similar to f2 and the other RNA phages and seems to be liberated by bacteriolysis. fr, due to the chemical nature of its nucleobases, is highly sensitive to hydroxylamine in slightly alkaline solution. Infection with fr in rare cases is non-lytic and leads to an unstable carrier state.

Der kleine, einsträngige DNS-Phage fd ist ein schlankes, flexibles Stäbchen und verkörpert durch seine Form einen neuen Typus von Bakteriophagen. Die folgenden Untersuchungen zeigen, daß der Phage durch seine biologischen Eigenschaften nicht weniger auffällt als durch seine Form. Der kleine RNS-Phage fr ist sphärisch; er ähnelt f2 $^1$ und den andern bekannten RNS-Phagen $^3$ $^4$. Die Reinigung der Phagen, ihre chemischen und ihre physikalischen Eigenschaften sind in den voraufgehenden Arbeiten beschrieben worden $^4$ $^5$.

**Material und Methoden**

Medien und Techniken waren die gleichen wie früher angegeben $^4$, Synthetisches Medium enthält (in mol/l): Tris-HCl-Puffer pH 7.4 0.1; KCl 2.7·10$^{-2}$; NH$_4$Cl 3.7·10$^{-2}$; MgCl$_2$ 2.5·10$^{-2}$; Na$_2$HPO$_4$ 5.0·10$^{-4}$; Na$_2$SO$_4$ 1.4·10$^{-4}$; CaCl$_2$ 1·10$^{-3}$; FeSO$_4$ 2.10$^{-6}$; Glucose 1·10$^{-2}$.

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