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Hydrodynamic Determination of Molecular Weight, Dimensions, and Structural Parameters of Pf3 Virus†

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ABSTRACT: Measurements of the translational, D_T, and rotational, D_R, diffusion coefficients of Pf3 virus by low-angle polarized intensity fluctuation spectroscopy and field-free transient electric birefringence, respectively, give a length of 720 ± 25 nm and diameter of 6.5 ± 1.5 nm upon simultaneous solution of the Broersma equations for rigid rods. Sedimentation coefficient and density increment values obtained under solvent conditions identical with those of D_T give a molecular weight of (13.4 ± 0.8) × 10^6 g mol^{-1}, which gives a mass per length of 18 600 ± 1300 g mol^{-1} nm^{-1}. Combining these results with the molecular weight of Pf3 DNA yields a number of protein subunits of 2500 ± 160 and 2.38 ± 0.14 nucleotides/protein subunit. Sedimentation coefficient and density increment values of Xf virus when combined with a value for the Xf translational diffusion coefficient [Chen, F. C., Koopmans, G., Wiseman, R. L., Day, L. A., & Swinney, H. L. (1980) *Biochemistry 19*, 1373] yield a molecular weight of (17.9 ± 1.0) × 10^6 g mol^{-1}, a number of protein subunits of 3590 ± 230, 2.07 ± 0.15 nucleotides/protein subunit, and a mass per length of 18 300 ± 1200 g mol^{-1} nm^{-1}. Thus, despite major differences in the DNA–protein packing between these viruses, as well as fd virus, the mass per lengths are surprisingly similar.

Structural data that have been gathered on a group of filamentous viruses, all of which have Gram-negative bacteria as hosts and all of which contain a circular single-stranded DNA molecule packed in a helical sheath of protein subunits, indicate considerable differences in the DNA structures maintained in the viruses, in the nature of chemical contacts between DNA and protein, and in the way differing symmetries of DNA and protein are meshed [Day & Wiseman (1978) Day et al. (1979), Marzec & Day (1980), Banner et al. (1981) Makowski & Caspar (1981), Nave et al. (1981), and Casadevall & Day (1982) are recent papers describing comparative data and current ideas]. The structural differences are striking in view of close similarities in overall morphology and in mass per length and the common function of packing single-stranded circular DNA. One of the main structural tools is X-ray fiber diffraction, but proper interpretation of fiber patterns is critically dependent on external input such as the size of the structural units and the mass per length. Indeed the currently accepted 5-fold rotational symmetry for fd (Makowski & Caspar, 1978, 1981) became apparent after the mass per length of fd was accurately determined (Newman et al., 1977). X-ray fiber studies are now underway on Pf3 virus (W. Winter, personal communication), and mass per length values are needed for that study. In addition, currently available data suggest that Pf3, like fd, may have a noninteger number of nucleotides per subunit, and establishment of this is also critical for structure studies. For these reasons an attempt was made to obtain independent measures of the mass, the mass per length and, through them, the chemical stoichiometry of Pf3 virus by carrying out measurements of its hydrodynamic properties. We have also gathered density and sedimentation velocity data on Xf virus, which we combine with the diffusion coefficients and hydrodynamic dimensions established by Chen et al. (1980). The

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results allow direct comparisons between fd, Pf3, and Xf structure parameters obtained by the same techniques, as well as by other techniques.

Hydrodynamic dimensions for Pf3 were obtained from the simultaneous solutions of two equations relating the measured rotational and the translational diffusion coefficients to the length and diameter of rigid rods. Two independent expressions for these equations are available: the Broersma relations (1960a,b, 1981), which were used in previous studies (Newman et al., 1977; Chen et al., 1980), and the results of the Tirado & Garcia de la Torre (1979, 1980) hydrodynamic calculations. The two sets of relations result in small differences in the hydrodynamic length of Pf3 but rather large differences (~30%) in the hydrodynamic diameter, a parameter that enters only logarithmically in both sets of relations and that is difficult to obtain experimentally by independent methods. A reinvestigation of previous results for fd and Xf virus indicates parallel differences in hydrodynamic lengths and diameters for these rodlike viruses as well. The major results and conclusions of previous work as well as those of the current study are not significantly affected by the differences in these two expressions.

Experimental Procedures

(*a*) *Preparation of Samples.* Pf3 virus was propagated on its host strain of *Pseudomonas aeruginosa* PA01 (RP1) bearing the RP1 plasmid that determines, in part, its host range (Stanisich, 1974). Like other filamentous viruses of Gram-negative bacteria, Pf3 does not lyse its host but is extruded into the growth medium. Our preparations were initially concentrated from the medium by precipitation through the addition of poly(ethylene glycol) to 20 g/L and NaCl to 0.5 M. The precipitates were dissolved in 5 mM sodium tetraborate buffer and subjected to two or more cycles of differential sedimentation involving clarifying spins at 17000*g* for 20 min and pelleting spins at 30000*g* for 3 h. Virus solutions in the borate buffer made from the pelleted material were brought to a density of 1.28 g cm^{-3} through the addition of CsCl and subjected to equilibrium banding in the density gradient formed by centrifugation at 45000*g* for 18 h. The principal virus band was recovered by draining the cellulose nitrate tubes from the bottom, and CsCl was removed by dialysis into the 5 mM borate buffer or into 15 mM sodium citrate. Samples used for determinations of the density increment and sedimentation coefficient were brought into 0.15 M NaCl–0.015 M sodium phosphate, pH 7.0, by dialysis. Samples for the rotational and translational diffusion coefficient measurements were purified further by velocity sedimentation of a band of virus through a preformed density gradient ranging from 20% to 5% sucrose. Centrifugation at 40000 rpm in a Beckman SW41 rotor for 180 min moved the virus band two-thirds of the way down the tube; it was fractionated on draining, and a peak fraction containing about one-fourth the material was recovered and freed of sucrose by dialysis against distilled water. Transfers into buffers used for the diffusion measurements were by dilution or dialysis.

Xf virus samples were prepared for sedimentation velocity measurements exactly as those prepared for the earlier diffusion studies (Chen et al., 1980), the procedure being quite similar to that just described for Pf3. The host for Xf is *Xanthamonas oryzae* (Kuo et al., 1969). Xf samples used for density increment measurements, seven in all, were prepared without sucrose gradient velocity sedimentation.

Concentrations were determined by absorbance measurements with extinction coefficients of 4.5 mg^{-1} cm^2 at 266 nm for Pf3 and 3.52 mg^{-1} cm^2 at 262 nm for Xf. Each extinction coefficient was based on an assumed value of 0.180 g^{-1} cm^3 for the refractive index increment at 546 nm in 150 mM NaCl–15 mM sodium phosphate, pH 7, and each was obtained through simultaneous measurements, in an analytical ultracentrifuge, of the refractive index difference and the absorbance difference across the boundary between the sedimenting virus and bulk solvent (Berkowitz & Day, 1980).

(*b*) *Density Increment.* Solution densities were determined with a Mettler-Parr digital densimeter, DMA02, with the measuring cell thermostated at 25.0 °C. So that uncertainties in the difference density measurement that might be introduced by unintended slight differences in the handling of the buffer sample and the virus solution sample could be eliminated, the procedure for dialysis and subsequent sample handling was as follows. One cubic centimeter of each virus solution, at a concentration between 0.5 and 3 mg cm^{-3}, was pipetted into a 7-mm diameter Visking dialysis bag, which was tied closed with a small air bubble inside. An identical bag was prepared with 1 cm^3 of the buffer, 0.15 M NaCl–0.015 M sodium phosphate, pH 7.0. The dialysis tubing had been prepared by boiling in Na_2CO_3 solutions and in ethylenediaminetetraacetic acid (EDTA) solutions, followed by thorough rinsing and soaking in distilled water; final rinses were with H_2O filtered through 0.2-μm pore filters. The two bags were placed in a 250-cm^3 bottle, which was sealed and then inverted once every 5 min by a rotary mixer on which it was mounted. Tests showed that equilibrium of diffusible components was achieved within 2 h, and dialysis was continued for at least 2 h each against two or more changes of outer solution. Solutions were withdrawn by syringe or pipet from the bags through small holes while the bags were still suspended in the outer dialysis solution and then placed in 1.5-cm^3 polypropylene centrifuge tubes, sealed, and centrifuged at 10000*g* for 4 min. Samples were then introduced by syringe into the clean, dry cell of the density meter. Each virus solution at each concentration and its parallel buffer sample were handled this way, with a serious attempt made to handle each of the pairs in identical manners. The solutions were allowed 20 min to come to thermal equilibrium, and then density readings were taken in the usual manner (Kratky et al., 1973). Concentrations were determined on the samples by absorbance measurements in 1–5-mm path optical cells without dilution.

The virus species for which the density increments hold, and hence for which molecular weights are determined, are defined through the concentration determination (Casassa & Eisenberg, 1964). For Pf3 and Xf, the values are for species having the assumed $(dn/dc)^0_\mu$ value of 0.180 g^{-1} cm^3 at 546 nm used to obtain concentrations. Many values for anhydrous proteins in similar solvents fall near 0.185 g^{-1} cm^3 [see Timasheff (1970)], and that for anhydrous DNA is near 0.163 g^{-1} cm^3 (Newman et al., 1977) so that viruses like Xf and Pf3 containing about 14% DNA (see below) might be expected to have a value of 0.182 g^{-1} cm^3; the value for the anhydrous reference state of the similar virus fd is 0.178 g^{-1} cm^3 (Berkowitz & Day, 1980). Thus, we consider the assumed $(dn/dc)^0_\mu$ value to define the concentrations of anhydrous species and to be numerically correct to within about 2%.

(*c*) *Sedimentation.* A Beckman Model E analytical ultracentrifuge equipped with photometric scanner detector and multiplexer was used. Pf3 samples were in the concentration range 40–300 μg cm^{-3}, and Xf samples were in the range 50–90 μg cm^{-3}. Virus solutions and reference buffer solutions were placed in the two sectors of the usual 12-mm path length, double-sector centrifuge cells. Three such cells were placed in an ANF rotor for each run and allowed several minutes to

come to thermal equilibrium. On most runs, the rotor was warmed to 25 °C from the usual ambient temperature near 23 °C. The rotor was then accelerated to either 36000 or 40000 rpm, and data were collected without thermoregulation, temperature drift being less than 0.3 °C during the runs, which lasted about 1 h. The radial positions of the sharp virus boundaries were measured at half the plateau heights in scans taken at various times. The sedimentation coefficients, s, were calculated from the least-squares slope of the logarithm of radial position vs. time. Eight to thirteen points per sample were measured, and temperatures were in the range 23–26 °C. For Pf3 the average least-squares slope for three plots of s vs. concentration, one with six points at 25 °C and two with three points each at temperatures near 25 °C, was -5.0 ± 0.8 s^{-13} mg^{-1} cm^3. This value was used to make minor corrections of s_{25} to s^0_{25} for two samples run only at 70 μg cm^{-3}. Unpublished results of F. C. Chen and of R. L. Wiseman (personal communication) indicate a negligible concentration dependence of s for Xf at temperatures near 25 °C, so observed s_{25} values were taken as s^0_{25} values.

(*d*) *Translational Diffusion.* The translational diffusion coefficient, D_T, was determined by low-angle polarized intensity fluctuation spectroscopy. The apparatus used was similar to a previously described system (Newman et al., 1974). The light source was the 488.0-nm TEM_{00} beam from an intensity-stabilized Ar^+ laser with a power of 10–50 mW incident on the samples. A temperature-controlled water bath (±0.05 °C) had an entrance $^1/_2$-in. optical window and a section of an optically polished hemisphere (1.855-in. inner and 2.183-in. outer radii), which allowed observation of scattered light at any angle between 0 and 105°. The bath was aligned so that the center of the hemisphere was accurately positioned on the axis of rotation of the collection optics. Calibration of the system with 0.109-μm polystyrene latex spheres (filtered through a 0.22-μm Millipore filter) indicated that a 1% accuracy in the translational diffusion coefficient could be obtained over a range of scattering angles from 8 to 105°.

Virus samples were prepared for the light scattering experiments by centrifugation at 30000g at 4 °C for 2 h. The samples were then transferred into optical cells in a positive-pressure dust-free box; the cells and pipets were cleaned inside a special acetone refluxing apparatus. The filled stoppered cells were then centrifuged overnight at 1500g to reduce the level of any residual dust.

Measurements were made at three to four scattering angles in the range 10–20° for each sample. The solvent was 150 mM NaCl–15 mM sodium phosphate, pH 7.0, and the measurements were performed at 25.00 ± 0.05 °C. A total of about 80 experiments, each representing 30–50 10-s experiments, was run. The data were scrutinized for the effect of dust on each correlation function, as determined from the average count rate and the size of the normalized second-order cumulant parameter (Koppel, 1972), and data affected by dust were discarded. The data were analyzed by using the theory for rigid rods in solution (Pecora, 1964). After the correction for rotational diffusion was made (Newman et al., 1974; Chen et al., 1980), the resulting translational diffusion coefficients were found to be independent of scattering angle over the range of angles studied.

(*e*) *Rotational Diffusion.* The rotational diffusion coefficient, D_R, was determined from an analysis of field-free transient electric birefringence. The apparatus has been described previously (Elias & Eden, 1981). Sample orientation was achieved with alternating unipolar pulses of 2.5-ms duration and electric fields of 185 V cm^{-1}. The resulting birefringence signal was averaged, typically 300 times, to enhance the signal-to-noise ratio. The field-free decay of the light intensity transmitted through the optics is a linear function of the birefringence and is related to the rotational diffusion coefficient of the virus by

$$I(t) = A \exp(-6D_R t) + B \qquad (1)$$

for a monodisperse sample. The intensity was fit to eq 1 by using a second-order cumulant analysis with the background B taken as the experimental average of the long-time intensity [$t \gg (6D_R)^{-1}$].

Three different Pf3 samples (1 mg cm^{-3} in 5 mM borate buffer) were diluted with sodium phosphate buffer, pH 7.0, to obtain the required concentrations (2 and 8 μg cm^{-3}) and ionic strengths (1 and 10 mM). The samples were spun at 5000g for 1 h to remove particulates, transferred to the birefringence cell, and degassed before a run commenced. All measurements were made at 24.75 ± 0.05 °C, and the results were corrected to 25.0 °C.

Chen et al. (1980) found in their study of Xf virus prolonged pulsing on the application of high electric fields irreversibly changes the birefringence signal. Therefore repeated measurements on a sample were examined for systematic trends in the relaxation time and degradation artifacts that might appear in the birefringence growth curve. Such artifacts could be readily discerned, and those data were excluded.

Results

Chen et al. (1980) showed in an electron microscopy (EM) study of their Xf samples that the separation techniques that we have used result in samples that are virtually free of multiple-length species and have narrow length distributions. Although we have not carried out a parallel EM study of our Pf3 preparations, the various measurements we have performed indicate a high degree of monodisperisity. The results are summarized in Table I.

(*a*) *Translational Diffusion.* The results of measurements on three virus preparations at concentrations ranging from 45 to 830 μg cm^{-3} are given in Table II. Each D_T value given represents results from data at typically three to four scattering angles in the range 10–20° after a small correction for rotational diffusion (see section d under Experimental Procedures). After this correction the apparent D_T values at each concentration showed no systematic variation with scattering angle and were averaged to obtain the results in Table II. Since the birefringence experiments required the use of a low ionic strength solvent, the dependence of the translational diffusion coefficient on ionic strength was checked in one set of measurements on a 45 μg cm^{-3} sample in a 10× dilution of the standard solvent. A weighted linear least-squares extrapolation of the diffusion coefficients in Table II to zero concentration yields $D^0_{T,25} = (3.33 \pm 0.06) \times 10^{-8}$ or $(3.29 \pm 0.06) \times 10^{-8}$ cm^2 s^{-1} if the low ionic strength value is not included, while a straight average of the values yields $(3.27 \pm 0.06) \times 10^{-8}$ cm^2 s^{-1}. Thus within our ~2% uncertainty we see no difference in the translational diffusion coefficient of Pf3 in these two solvents.

(*b*) *Rotational Diffusion.* The values of the apparent rotational diffusion coefficients for our three Pf3 samples are given in Table III. In 1 mM phosphate buffer we observe that the D_R values do not exhibit any concentratioin dependence. This is the result we expect since the interparticle spacing is at least twice the length of the virus (they are equal at 50 μg cm^{-3}). Sample 4 systematically exhibits a greater $D_{R,25}$ than the other two samples. The 8% larger D_R could

Table I: Summary of Properties of Pf3 Virus in Solution

measured	
translational diffusion coeff, $D^0_{T,25}$ [a] ($cm^2\ s^{-1}$)	$(3.30 \pm 0.10) \times 10^{-8}$
rotational diffusion coeff, $D^0_{R,25}$ [b] (s^{-1})	47.4 ± 1.4
sedimentation coeff, s^0_{25} [a] (S)	$(42.0 \pm 1.2) \times 10^{-13}$
density increment, $(\partial\rho/\partial c)^0_\mu$ [a,c]	0.235 ± 0.009
derived	
hydrodynamic length, L [d] (nm)	720 ± 25
hydrodynamic diam, d [d] (nm)	6.5 ± 1.5
mol wt of virus [e] ($g\ mol^{-1}$)	$(13.4 \pm 0.8) \times 10^6$
mass/length [d] ($g\ mol^{-1}\ nm^{-1}$)	$1.86 \pm 0.13) \times 10^4$
no. of protein subunits [f]	2500 ± 160
nucleotides/protein subunit	2.38 ± 0.14
nucleotide axial spacing [g] (nm)	0.242 ± 0.009
protein subunit axial spacing [g] (nm)	0.288 ± 0.019
hydration [h] (cm^3 of solvent/g of virus)	0.31 ± 0.14

[a] Measured in 150 mM NaCl–15 mM sodium phosphate, pH 7.0 at 25 °C. [b] Measured in 1 mM sodium phosphate, pH 7.0 at 25 °C (see Table III and section b under Results). [c] The solvent density is 1.0060 g cm^{-3}, which yields an apparent specific volume ϕ' of $0.760 \pm 0.009\ g^{-1}\ cm^3$. [d] Based on the Broersma relations (see Figure 1 and section a under Discussion). The Tirado and Garcia de la Torre relations yield $L = 707 \pm 25$ nm and $d = 8.3 \pm 1.9$ nm. [e] From the Svedberg equation and the measured values of s^0, D^0_T, and $(\partial\rho/\partial c)^0_\mu$ at the same temperature and solvent conditions. The value is for the virus species defined by the method of concentration determination (see section b under Experimental Procedures). [f] Calculated by using a molecular weight of a protein subunit of 4632 g mol^{-1} (D. Gluck Putterman and B. Frangione, personal communications) and a value of 5960 ± 90 nucleotides, each of 308 g mol^{-1} average mass [Berkowitz & Day, 1980; an independent determination by a restriction enzyme mapping of the Pf3 genome is in agreement with this value (D. Putterman, personal communication)]. According to the sodium dodecyl sulfate gel electrophoresis of the purified virus (with Coomassie Blue staining), the fraction of minor coat protein is similar to that of fd (2–3%; L. A. Day, unpublished results). In the calculations here, however, we have chosen to ignore this small and uncertain correction. [g] Average spacing along each up or down strand of the circular DNA in the virus. [h] Calculated from the ratio of the difference between the hydrodynamic volume ($\pi d^2 L/4$) and the "dry" volume ($\phi' M/N_A$, where N_A is Avogardo's number) to the mass of a virus particle (M/N_A). If the virus dimensions obtained from the Tirado and Garcia de la Torre equations are used here, then the hydration is found to be 0.96 $cm^3\ g^{-1}$.

Table II: Translational Diffusion Coefficients of Pf3 Virus in 0.15 M NaCl–0.015 M Sodium Phosphate, pH 7.0 at 25 °C

sample	concn ($\mu g\ cm^{-3}$)	z-av diffusion coeff, [a] D_T ($10^{-8}\ cm^2\ s^{-1}$)
2	130	3.32
	450	3.27
3	100	3.19
	830	3.09
4	45 [b]	3.37
	110	3.39
	230	3.24

[a] Obtained by the method of cumulants (Koppel, 1972). The values given represent the average of the angle-independent results after a small correction for rotational diffusion (see section d under Experimental Procedures). [b] The solvent for these data was 15 mM NaCl–1.5 mM sodium phosphate, pH 7.0 at 25.0 °C.

result from an average decreased length of 3%. Given that there may be differences in each preparation and that the virus is susceptible to shear, we believe that the measurements on samples that exhibit the smallest D_R values are the most reliable. Examination of Table III also shows that the rotational diffusion coefficient in low salt is approximately 10% less than in the 10 mM buffer. Since the electrostatic persistence length

Table III: Rotational Diffusion Coefficients of Pf3 Virus in Sodium Phosphate Buffer, pH 7.0 at 25 °C

sample	ionic strength (mM)	concn ($\mu g\ cm^{-3}$)	$D_{R,25}$ (s^{-1})
1	1	2	47.5
	1	8	47.0
2	1	2	47.3
	1	8	47.7
	10	8	52.8
4	1	8	51.6
	10	8	56.6

Table IV: Density Increment Data for Pf3 and Xf Viruses in 0.15 M NaCl–0.015 M Sodium Phosphate, pH 7.0 at 25 °C

	concn ($mg\ cm^{-3}$) [a]	$(\Delta\rho/\Delta c)_\mu$
Pf3-A	1.8 (1)	0.227
	2.7 (2)	0.231
Pf3-B	1.2 (1)	0.262
Pf3-C	0.6 (1)	0.245
	0.9 (3)	0.232
	1.8 (4)	0.233
average value for Pf3		0.235 ± 0.009
Xf-A	1.8, 3.5	0.222
Xf-B	2.0, 3.7, 4.9, 7.3	0.242
Xf-C	0.7, 1.5, 2.5	0.233
Xf-D	0.8, 1.3, 1.8	0.227
Xf-E	2.0 (3)	0.234
Xf-F	2.6 (2)	0.241
Xf-G	2.6 (2)	0.250
average value for Xf		0.236 ± 0.008

[a] Data for the same preparation at concentrations within 0.1 mg cm^{-3} have been grouped and averaged together; the number of sample and buffer pairs measured is given in parentheses.

increases with decreasing salt concentration we may be observing a limited amount of flexibility of the Pf3 virus at higher salt. The transient birefringence experiments are much more sensitive to small changes in effective length than are the quasi-elastic light scattering or sedimentation experiments. For our hydrodynamic calculations we use the low-salt values of D_R since they are most likely to give the rigid rod limit; however, the hydrodynamic length that we obtain has an uncertainty range, which includes the value that would be obtained from the value of D_R in 10 mM salt.

(*c*) *Sedimentation and Density Increment.* The sedimentation coefficients for six different Pf3 virus preparations in 0.15 M NaCl–0.015 M sodium phosphate at pH 7.0 corrected to 25.0 °C and to the zero concentration limit, as described in section b under Experimental Procedures, gave an average s^0_{25} of 42.0 ± 1.2 S. One of the six individual values was established from six concentration points and linear least-squares extrapolation to zero concentration limit; the other five values were from runs in the range 40–70 $\mu g\ cm^{-3}$, corrected to zero concentration. The stated uncertainty is the standard deviation of the six values about their mean.

The results of the density increment measurements for Pf3 are compiled in Table IV. As for the hydrodynamic measurements, different virus preparations were used. There is no indication of concentration dependence, so the simple average value of 0.235 ± 0.009 (dimensionless) was taken for $(\partial\rho/\partial c)^0_\mu$, the density increment at infinite dilution and chemical equilibrium of all diffusible components. The density of the buffer is $\rho = 1.0060\ g\ cm^{-3}$, so the value of the apparent partial specific volume (Casassa & Eisenberg, 1964) in the relation $(\partial\rho/\partial c)^0_\mu = 1 - \phi'\rho$ is ϕ' in $0.760 \pm 0.009\ g^{-1}\ cm^3$.

Our value of the partial specific volume for Pf3 is about 5% greater than the value of $0.720 \pm 0.008\ g^{-1}\ cm^3$ found for fd

virus (Newman et al., 1977) in 0.15 M KCl–0.015 M sodium phosphate. A determination for fd in the buffer used for Pf3 and Xf gave 0.718 ± 0.005 g^{-1} cm^3.

We also report here the sedimentation coefficient of Xf virus. The average value obtained from four different virus preparations in the same solvent used in the Pf3 measurements was 43.3 ± 1.1 S. The average of 21 determinations of the density increment of Xf virus in the same buffer as the Pf3 work results in a value of $(\partial\rho/\partial c)^0_\mu = 0.236 \pm 0.010$, so that $\phi' = 0.759 \pm 0.010$ for Xf virus, in agreement with the value of Pf3 virus. The small but significant differences between the partial specific volume for fd and that for either Xf or Pf3 may lie in real differences in the structures and solvation of the viruses.

Discussion

(*a*) *Dimensions and Hydration of Pf3 Virus.* There are two pairs of calculations in the literature that relate D_T and D_R each to the length, L, and diameter, d, of a rigid rod in solution (Broersma, 1960a,b, 1981; Tirado & Garcia de la Torre, 1979, 1980). The Broersma equations

$$D_R = \frac{3k_BT}{\eta\pi L^3}\left(\ln\frac{L}{d} - \gamma_R\right) \quad (2)$$

and

$$D_T = \frac{k_BT}{3\pi\eta L}\left(\ln\frac{L}{d} - \gamma_T\right) \quad (3)$$

where k_B is the Boltzmann constant, η is the solvent viscosity, and with end-effect correction terms

$$\gamma_R = 0.757 - 7.5\left[\frac{1}{\ln(2L/d)} - 0.27\right]^2 \quad (2a)$$

and

$$\gamma_T = -5.8\left[\frac{1}{\ln(2L/d)}\right]^2 + 4.15\left[\frac{1}{\ln(2L/d)}\right] - 0.71 \quad (3a)$$

have been previously used to solve for both L and d of the filamentous viruses fd (Newman et al., 1977) and Xf (Chen et al., 1980) from measurements of D_T and D_R. Figure 1 shows the appropriate plot of the Broersma equations in the current study. Our values of D_T and D_R yield the length and diameter values of 720 ± 25 nm and 6.5 ± 1.5 nm, respectively (see Table I). The value for the hydrodynamic length is 5% greater than the length determined by electron microscopy by Berkowitz & Day (1980) and 6% less than an earlier EM length determination (Bradley, 1974). As indicated in sections a and b under Results, the diffusion coefficient may indicate some small degree of flexibility, but, if present, this effect would reduce the hydrodynamic length by at most ~3% and increase the hydrodynamic diameter by ~10%.

The model of Tirado & Garcia de la Torre was used in a similar way to extract a value for L and d from our results. Their calculations, based on modeling rods as stacked rings of spheres, result in different correction terms

$$\gamma_R = 0.662 - 0.92(d/L) \quad (2b)$$

and

$$\gamma_T = 0.313 - 0.601(d/L) \quad (3b)$$

Equations 2b and 3b were obtained from linear fits to the large axial ratio data of Table II and Table I in Tirado & Garcia de la Torre (1980) and Tirado & Garcia de la Torre (1979), respectively. Their model yields a value of L = 707 ± 25 nm, 1.8% smaller than the value obtained from the Broersma equations, and a value of d = 8.3 ± 1.9 nm, considerably greater (~30%) than the Broersma value.

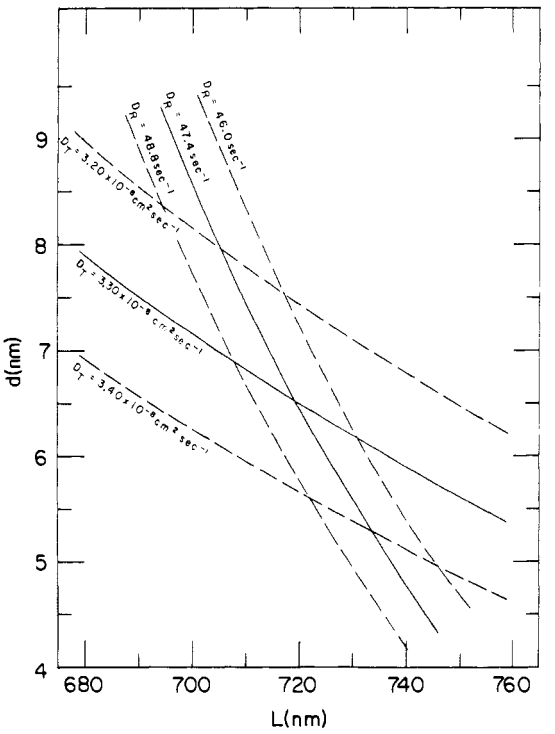

FIGURE 1: Simultaneous solution of the Broersma relations (see eq 2, 3, 2a, and 2b) for lengths and diameters of rigid rods with $D^0_{T,25}$ = (3.30 ± 0.10) × 10^{-8} cm^2 s^{-1} and $D^0_{R,25}$ = 47.4 ± 1.4 s^{-1}. The results for the hydrodynamic length and diameter are 720 ± 25 nm and 6.5 ± 1.5 nm, respectively. A similar solution to the Tirado and Garcia de la Torre equations, equations 2b and 3b, results in a hydrodynamic length and diameter of 707 ● 25 nm and 8.3 ± 1.9 nm, respectively.

The two calculations yield differences in the hydrodynamic length and diameter that are within our experimental uncertainties. The 30% difference in the hydrodynamic diameters obtained does not affect any of our primary results. In fact, the only derived quantity that is affected by the diameter of the virus is the hydration value in Table I [calculated as in Newman et al. (1977)], which is increased from 0.31 to 0.96 cm^3 of water/g of virus.

Tirado & Garcia de la Torre (1980) state that when the rod axial ratio is not too large their touching sphere model can lead to a significant overestimation of the calculated rotational diffusion coefficient (or, equivalently, given a value of D_R and d, their model would underestimate L). They also conclude [Table IV of Tirado & Garcia de la Torre (1979)] that given values for L and D_T, the Broersma equation underestimates the hydrodynamic diameter by ~25% for an axial ratio of 100.

In view of the large difference in the two values for the hydrodynamic diameter of Pf3 we calculated L and d values from a simultaneous solution of eq 2 and 3 for both fd and Xf virus on the basis of the published D_T and D_R values. In both cases the L values obtained were ~2% smaller and the d values were ~30% greater than the published values based on the Broersma equation.

As was discussed by Newman et al. (1977) and Chen et al. (1980), the hydrodynamic diameters calculated from the Broersma relations for fd and Xf virus are in agreement with both the electron microscopy and X-ray unit-cell dimensions for these virus particles. We consider it highly unlikely that the larger diameters calculated from the Tirado and Garcia de la Torre equations reflect thick boundary layers of hydrodynamically trapped water. Our results for the hydrodynamic lengths of these virus particles do not allow us to select the Broersma or Tirado and Garcia de la Torre expressions.

Table V: Structural Parameters of Pf3, fd, and Xf Derived from Hydrodynamic Properties

	Pf3	fd[a]	Xf
length (nm)	720 ± 25	895 ± 20	980 ± 30[b]
mass (10^6 g mol^{-1})	13.4 ± 0.8	16.4 ± 0.6	17.9 ± 1.0
mass per length (10^4 g mol^{-1} nm^{-1})	1.86 ± 0.13	1.83 ± 0.08	1.83 ± 0.12
no. of protein subunits	2500 ± 160	2750 ± 100	3590 ± 230
no. of nucleotides	5960 ± 90	6408[c]	7420 ± 240[d]
nucleotides/protein subunit	2.38 ± 0.14	2.33 ± 0.08	2.07 ± 0.15
protein axial translation (nm)	0.288 ± 0.019	0.325 ± 0.014	0.272 ± 0.019
nucleotide axial translation (nm)	0.242 ± 0.009	0.279 ± 0.006	0.264 ± 0.006

[a] From Newman et al. (1977). [b] From Chen et al. (1980). [c] fd DNA has been sequenced by Beck et al. (1978). [d] From Wiseman & Day (1977). Note: The mass per (EM) length of Pf1 virus calculated from data summarized by Day & Wiseman (1978) is 1.86×10^4 g mol^{-1} nm^{-1} with the assumption of 1.0 nucleotide/subunit; also, the mass per (EM) length of Xf based on exactly 2 nucleotides/subunit is 1.88×10^4 g mol^{-1} nm^{-1}.

Without additional independent values for the hydrodynamic diameters, we can only indicate that significant differences result from the two calculations and urge caution in applying them.

(*b*) *Molecular Weight and Structural Parameters.* The values for the sedimentation and translational diffusion coefficients and the density increment, all measured in the same solvent, can be combined in the Svedberg equation to yield molecular weight values. For Pf3 virus we obtain (13.4 ± 0.8) × 10^6 g mol^{-1}. From the D_T value for Xf virus (Chen et al., 1980) and the s and $\partial\rho/\partial c$ results of this study, all measured in the same solvent, the molecular weight of Xf is, similarly, found to be (17.9 ± 1.0) × 10^6 g mol^{-1}. These values, when divided by the corresponding hydrodynamic lengths, yield mass per length values of 18 600 ± 1300 and 18 300 ± 1200 g mol^{-1} nm^{-1} for Pf3 and Xf, respectively, in agreement with each other and with the corresponding value of 18 300 ± 800 g mol^{-1} nm^{-1} obtained from similar data on fd virus (Newman et al., 1977). The mass per length values obtained in these hydrodynamic studies are 5.4%, 4.4%, and 4.4% lower than the corresponding values obtained for Pf3, Xf, and fd, respectively, from turbidity measurements in an ultracentrifuge (Berkowitz & Day, 1980). Thus, despite apparent small systematic differences between the hydrodynamic and turbidometric results, each technique finds that the mass per length of the three viruses are, within experimental uncertainty, equal.

Using values for the molecular weight of Pf3 DNA and Xf DNA of (1.84 ± 0.06) × 10^6 and (2.29 ± 0.07) × 10^6 g mol^{-1}, respectively, based on an average nucleotide mass and the number of nucleotides, we find that Pf3 and Xf virus consist of 13.9 ± 1.0% and 12.8 ± 0.8% DNA. These results agree with values of 14% for Pf3 (Berkowitz & Day, 1980) and 13% for Xf (Wiseman & Day, 1977).

Values for other structural parameters of Pf3 and Xf virus can be derived by combining our results with values for the mass of a protein subunit, the number of nucleotides in the DNA, and the average nucleotide mass. By subtracting the DNA molecular weight from the virus molecular weight and dividing the result by the protein subunit mass, we find there are 2500 ± 160 and 3600 ± 230 protein subunits in Pf3 and Xf, respectively. These result in a nonintegral value of 2.38 ± 0.14 for the number of nucleotides per protein subunit in Pf3 virus, whereas the corresponding number of 2.06 ± 0.15 obtained for Xf virus may reflect an exact integral ratio of nucleotides per subunit [see Day & Wiseman (1978)].

Table V summarizes the derived structural properties of Pf3 and Xf as well as those of fd virus. We find it remarkable that three viruses with such different parameters should all have the same mass per length. It is worth noting that a fourth filamentous virus, Pf1, which has 1.0 nucleotide/protein subunit, also has a calculated mass per length (EM) of 18 600 g mol^{-1} nm^{-1}. The significance of the equal linear mass densities is not presently known. Table V also shows that Pf3 and fd both have nonintegral nucleotide to protein subunit ratios, which are nearly the same, and Pf3 and Xf have equal protein axial translations. Notice that no two viruses have, in addition to mass per length, more than one other structural parameter in common. Makowski & Caspar (1981) have discussed some aspects of symmetry differences in filamentous virions, and Nave et al. (1981) have noted mass per length similarities on the basis of unit-cell dimensions. C. J. Marzec and L. A. Day (1980; unpublished results) have recently suggested that such similarities for Pf1 and fd are due to geometric constraints imposed by the stable packing arrangement of protein subunits and nucleotides, and they have used these physicochemical results to develop structural models. The results of this study provide additional information for use in establishing the structure of filamentous viruses.

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Rapid-Quench and Isotope-Trapping Studies on Fructose-1,6-bisphosphatase†

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ABSTRACT: Rapid-quench kinetic measurements yielded pre-steady-state rate data for rabbit liver fructose-1,6-bisphosphatase (FBPase) (a tetramer of four identical subunits) that are triphasic: the rapid release of P_i (complete within 5 ms), followed by a second reaction phase liberating additional P_i that completes the initial turnover of two or four subunits of the enzyme (requiring 100-150 ms), and a steady-state rate whose magnitude depends on the [α-Fru-1,6-P_2]/[FBPase] ratio. With Mg^{2+} in the presence of excess α-fructose 1,6-bisphosphate (α-Fru-1,6-P_2) all four subunits turn over in the pre steady state; with Mn^{2+} only two of the four are active. Thus the expression of half-site reactivity is a consequence of the nature of the metal ion and not a subunit asymmetry. In the presence of limiting α-anomer concentrations only two of the four subunits now remain active with Mg^{2+} as well as with Mn^{2+} in the pre steady state. However, so that the amount of P_i released can be accounted for, a $\beta \rightarrow \alpha$ anomerization or direct β utilization is required at the active site of one subunit. Such behavior is consistent with the two-state conformational hysteresis displayed by the enzyme and altered affinities manifested within these states for α and β substrate analogues. Under these limiting conditions the subsequent steady-state rate is limited by the $\beta \rightarrow \alpha$ solution anomerization. These data in combination with pulse-chase experiments permit evaluation of the internal equilibrium, which in the case of Mg^{2+} is unequivocally higher in favor of product complexes and represents a departure from balanced internal substrate-product complexes.

Fructose-1,6-bisphosphatase (EC 3.1.3.11, D-fructose-1,6-bisphosphate 1-phosphohydrolase, FBPase[1]) (Benkovic & de Maine, 1982) catalyzes the hydrolysis of D-fructose 1,6-bisphosphate (Fru-1,6-P_2) to D-fructose 6-phosphate (Fru-6-P) and inorganic phosphate (P_i). It is a tetrameric protein with four identical subunits (Traniello et al., 1971) and requires Mg^{2+}, Mn^{2+}, Zn^{2+}, or Co^{2+} for its catalytic activity. Kinetic studies with Mn^{2+} on the rabbit liver enzyme demonstrated its high stereospecificity for the α anomer of Fru-1,6-P_2 (Frey et al., 1977). However, the stereospecificity appears anomalous in that the enzyme binds both the α and β anomers of methyl D-fructofuranoside 1,6-bisphosphate (K_i = 7.2 μM and 1.7 μM, respectively) (de Maine & Benkovic, 1972). An explanation is provided by the existence of hysteretic active and resting forms of FBPase (de Maine & Benkovic, 1979). Results from pre-steady-state kinetic investigations employing a phenol red indicator method on FBPase with Mg^{2+} fitted the proposed hysteresis. They suggested a reaction sequence in which binding of the substrate is followed by a first-order conformational change prior to the establishment of the steady state. The conformational change (manifest by proton release) is followed by a rapid proton uptake of a magnitude corresponding to the release of P_i from a molecule of Fru-1,6-P_2 by each subunit of the FBPase. After breakdown of the remaining substrate at the steady-state rate the catalytic cycle ends with the decay of the enzyme to its initial unreactive state (Benkovic et al., 1979).

The present study concerns itself with molecular events that occur in the transient phase, namely, (1) verification that P_i is indeed released in this phase, (2) the stoichiometry of P_i release relative to conditions where Fru-1,6-P_2 as the α anomer is less than FBPase subunit concentration, (3) the influence

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[1] Abbreviations: FBPase, fructose-1,6-bisphosphatase; Fru-1,6-P_2, fructose 1,6-bisphosphate; Fru-6-P, fructose 6-phosphate; P_i, inorganic phosphate; EDTA, ethylenediaminetetraacetic acid; $NADP^+$, nicotinamide adenine dinucleotide phosphate; Tris, tris(hydroxymethyl)aminomethane; NMR, nuclear magnetic resonance.

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