Dimensions of Xf Virus from Its Rotational and Translational Diffusion Coefficients†

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ABSTRACT: Measurements on monodisperse preparations of the filamentous virus Xf give 17.3 ± 0.6 s^{-1} for its rotational diffusion coefficient in 1 mM sodium phosphate, pH 7.5, at 20 °C and (2.53 ± 0.06) × 10^{-8} cm^2 s^{-1} for its translational diffusion coefficient in 0.15 M NaCl and 0.015 M sodium phosphate, pH 7, at 25 °C. These coefficients yield a length of 980 ± 30 nm and a diameter of 7.2 ± 1.5 nm for the virus in solution when used to solve simultaneously the two Broersma equations for the diffusion of rigid rods.

Xf bacteriophage infects *Xanthomonas oryzae*, a bacterium that causes leaf blight on rice plants (Kuo et al., 1967). It is one of several filamentous viruses having small circular single-stranded DNA genomes [for reviews see Marvin & Hohn (1969) and Day & Wiseman (1978)]. The original papers on Xf by Kuo and co-workers included measurements of images of about 100 virus particles in electron micrographs which gave a length of 977 nm and a diameter of about 8 nm (Kuo et al., 1969). X-ray diffraction patterns from oriented fibers of Xf virus show that the diameter is about 6 nm and that the structure has a periodicity of 7.7 nm with the protein subunits arranged in a helix of 1.5-nm pitch around the DNA core (Marvin et al., 1974). More recently the sequence of the 44 amino acids in the major coat protein has been established (Frangione et al., 1978), and values for the number of nucleotides in its DNA, 7400, and the total number of major coat protein subunits in the virion, 3700, have also been determined (Wiseman & Day, 1977; Day & Wiseman, 1978).

In this paper we show that Xf virus preparations contain end-to-end multimers which can be removed by fractionation procedures. We then describe measurements of rotational diffusion by transient electric birefringence and translational diffusion by intensity fluctuation spectroscopy on monodisperse samples. Following Newman et al. (1977), we have used Broersma's two equations relating the length and diameter of a rigid rod to the two diffusion coefficients to obtain the dimensions of Xf virus in solution. The results are discussed in terms of Xf virus structure and of hydrodynamic studies on other filamentous viruses.

Materials and Methods

Initial Preparation and Characterization of Samples. Xf virus isolated from a single plaque was grown and purified following the procedure of Kuo et al. (1969) as modified by Marvin et al. (1974). Concentrations were determined by absorbance measurements using an extinction coefficient of 3.52 mg^{-1} cm^2 at 263 nm (Day & Wiseman, 1978). Initial measurements by transient electric birefringence on virus prepared by two precipitations from 2% poly(ethylene glycol) and 0.5 M NaCl, followed by one equilibrium banding in a CsCl density gradient, gave rotational correlation times about 50% longer than expected on the basis of the contour length from electron microscopy (Kuo et al., 1969). This suggested either that the length in solution was about 15% longer than that given by electron microscopy or that a significant fraction of longer than normal virus particles were present in the preparations. Analytical agarose gel electrophoresis was carried out according to Herrmann et al. (1978) with 1% agarose vertical slab gels and Coomassie blue staining. As many as six distinct bands having mobilities of 0.18, 0.30, 0.45, 0.64, and 0.86 relative to the predominant band were observed. Both the rotational correlation times and the gel electrophoresis band patterns indicated the possible presence of multiple-length species in the standard unfractionated preparations. This was confirmed by electron microscopy (see below).

Fractionation Procedures. Preparative electrophoresis was done with the conditions used for analytical gels but the samples were applied in wells 12 cm wide. After electrophoresis, three thin vertical sections of the slab were stained and used to locate the main band in the unstained portion. This band contained the monomer length Xf virion. The gel matrix was gently crushed into small pieces and suspended in buffer, and virus was recovered by soaking the crushed agarose for 2 or 3 days at 4 °C. Virus so prepared migrated as a single band in analytical gels but had lower specific infectivity than virus not subjected to electrophoresis (less than 1% vs. ~20% infectious particles).

The second fractionation technique was sucrose density gradient velocity sedimentation. Linear gradients of 10–30% sucrose (w/v) in 50 mM Tris-HCl, pH 7.5, were prepared in tubes for an SW41 rotor of a Beckman preparative ultracentrifuge. Approximately 600 μg of Xf virus in 0.2 mL of 50 mM Tris buffer was applied to each tube. Centrifugation for 15 h at 28 000 rpm at 5 °C moved the absorbance peak approximately two-thirds of the way down each tube. The absorbance profiles appeared to result from a superposition of the absorbance from fast-sedimenting species upon the skewed absorbance profile expected for the principal component. Fractions of the material sedimenting at the peak of the absorbance profile for each tube were collected and pooled, as were fractions before and after these principal fractions. The specific infectivity in the three central fractions was similar to that of the starting material (~20% infectious particles).

Electron Microscopy. Length distributions of virus preparations before and after fractionation by gel electrophoresis or sucrose gradient centrifugation were established by electron microscopy. A drop of a virus solution containing 2 μg/mL

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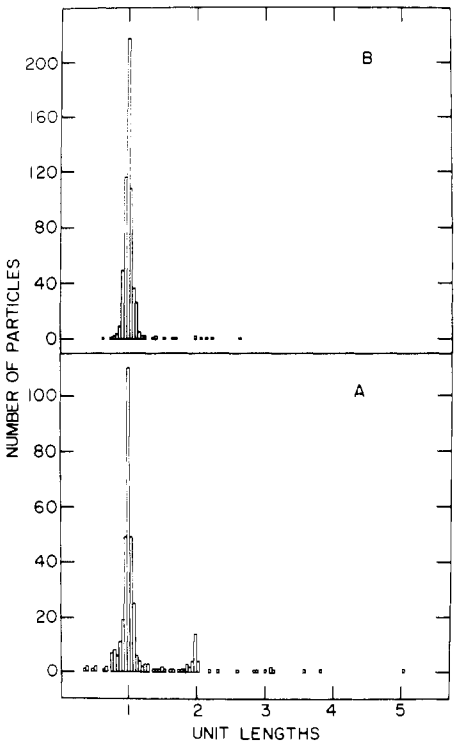

FIGURE 1: Length distribution of samples of Xf before (A) and after (B) separation of monomer length particles from multimers by agarose gel electrophoresis. For (A), 371 images obtained at a microscope magnification of 4700× were measured; for (B), 580 images obtained at 6000× were measured.

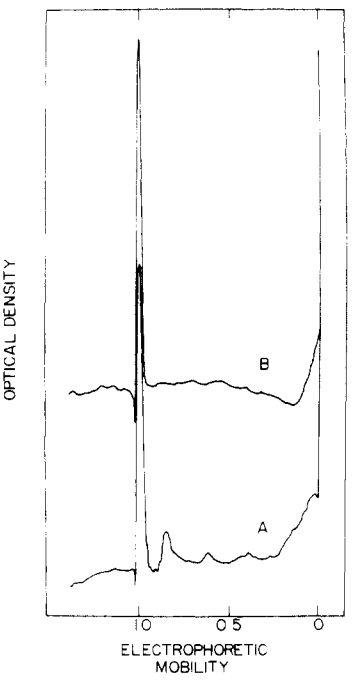

FIGURE 2: Densitometer traces of gels stained by Coomassie blue for a sample of Xf virus before (A) and after (B) separation of monomer length particles by sucrose gradient centrifugation. Electron microscopy of the sample characterized by trace B gave a distribution similar to that of Figure 1B with about 2% of the particles longer than unit length.

was placed on a carbon-coated grid. After a few seconds adsorption time, the droplet was drawn from the grid with filter paper. The grid was then washed with 2 or 3 drops of water and subjected to platinum shadowing at an angle of approximately 10° to the plane of grid. During shadowing, the grids were rotated in that plane for part of the time and then held stationary. Fields were photographed at microscope magnifications of 4700× and 6000×. Micrographs containing between 30 and 90 particles were magnified further on a photographic enlarger to give contour lengths of approximately 2.4 cm for images with the most frequently occurring length. The contour lengths of each particle were taken as the average of four measurements made with a Numonics digital planimeter, the statistical uncertainty in each average length being about 1%. After normalization for random variations (~10%) in overall magnification for different fields, the data for separation by gel electrophoresis were plotted in the histograms of Figure 1.

Multiples of the unit-length particles are evident in Figure 1A. In addition, there seem to be a few particles with lengths between the unit length and the dimer length. End-to-end multimers have been observed for filamentous viruses of *Escherichia coli* (Marvin & Hoffmann-Berling, 1963; Bradley, 1964; Beaudoin et al., 1974). In addition, Wheeler & Benzinger (1978) have shown that filamentous coliphage particles with lengths intermediate between the monomer and dimer probably have abnormal DNA packaging. The present data (Figure 1A) suggest that the occurrence of end-to-end aggregation and occasional abnormal DNA packaging might also be characteristic of the Xf virus system. As the purpose of the present study was to obtain dimensions from hydrodynamic measurements, these effects have not been investigated further. Comparison of parts A and B of Figure 1 shows the improvement in sample homogeneity achieved by agarose gel electrophoresis. We used both gel electrophoresis and electron microscopy to characterize the separation of multimers from monomers by sucrose gradient velocity sedimentation, as indicated in Figure 2.

Rotational Diffusion Measurements. The transient electric birefringence technique was used to determine the rotational diffusion coefficient. The instrumentation was that described by Newman & Swinney (1976), and its application to the filamentous virus Xf was similar to the application by Newman et al. (1977) to fd virus. The sample buffer was 1 mM sodium phosphate, pH 7.5. The external orienting field pulses ranged from 60 to 360 V cm^{-1} and were 10 ms in duration. The field-free decay of the light intensity transmitted through the optical system was related to the rotational diffusion coefficient, D_R, of the sample by

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

where A and B are constants (Newman & Swinney, 1976). The decay curves for many repeated pulses, of alternate sign to avoid electrophoresis, were signal-averaged by the multichannel analyzer. The decay times were independent of the magnitude of the applied field for less than 512 pulses, but even though the applied field strength was small, there was degradation of Xf virus not observed for fd. This problem could be circumvented by the use of fresh samples after every signal-averaging series (256–512 separate measurements) and low experimental temperatures (5–12 °C). The birefringence data were corrected to 20 °C by means of literature values for the temperature dependence of solvent viscosity according to the Stokes–Einstein relationship.

Translational Diffusion Measurements. The translational diffusion coefficient, D_T, was determined from measurements of light scattering intensity autocorrelation functions. The apparatus used was that described by Newman et al. (1974), and the measurements were at a scattering angle of 11.82° and a temperature of 25.0 ± 0.1 °C. The buffer was 0.15 M NaCl and 0.015 M sodium phosphate, pH 7.0. The laser beam power was approximately 20 mW and the wavelength was

488.0 nm. The light-scattering cells were polished quartz rectangular cells 10 × 10 × 40 mm. They were cleaned in a distillation apparatus which allowed freshly distilled acetone to flush the inside of the cell repeatedly. The virus samples were centrifuged at 10000*g* for 0.5 h and transferred to the clean cells by means of a peristaltic pump. The filled cells were centrifuged overnight at 1500*g* to reduce the level of any residual dust before being placed in the thermostat bath of the light-scattering instrument.

The effect of dust on each autocorrelation function was determined from the average count rate, the size of the autocorrelation function at large time delays, and the size of cumulant (Koppel, 1972). The data affected by dust were rejected. Thirty to one hundred correlation function measurements, each of 50-s duration, were made for each set of sample conditions.

The intensity autocorrelation function data were analyzed by using the theory for rigid rods in solution (Pecora, 1964) which gives for the normalized autocorrelation function

$$g^{(2)} - 1 = A[1 + (2B_2/B_0)\exp(-6D_R t) + ...]\exp(-2D_T q^2 t) \quad (2)$$

where B_2/B_0 vanishes as $qL \to 0$ (L = rod length; q = magnitude of the scattering vector). The higher order terms omitted from the expression in brackets make negligible contribution to $g^{(2)} - 1$ at the low scattering angle used for our measurements, and even the term with $2B_2/B_0$ as coefficient contributes at most only 2.5%. In principle, making the correction for the effects of rotational diffusion to obtain D_T requires an iterative procedure since B_2/B_0 depends on L and L is to be derived from both D_T and D_R. In this study the correction was so small that an initial estimate of L from an initial value of D_R gave single-exponential fits for $g^{(2)} - 1$ divided by the term in brackets and values of D_T within 0.3% of those the data would have yielded through another iteration cycle. The small difference indicated satisfactory convergence so the data were not reprocessed.

Results

Rotational Diffusion. Three different samples of Xf virus were used for measurements of D_R. Time-averaged decay curves for 18 sets of measurements at concentrations ranging from 2 to 20 $\mu g\ cm^{-3}$ were analyzed by subjecting the 400 points of each decay curve to a three-parameter fit to eq 1. The values of D_R obtained from all of the measurements are plotted vs. concentration in Figure 3. We are not certain that the apparent slight decrease in D_R with concentration is a true characteristic of the system. If there were in fact concentration dependence, then a least-squares fit of the data to $D_R = D_R^0 + (\partial D_R/\partial c)c$ would give $D_R^0 = 17.6\ s^{-1}$. If there were no concentration dependence, then $D_R^0 = \bar{D}_R = 17.1\ s^{-1}$. Under these circumstances, we think that a value for D_R^0 of 17.3 ± 0.6 s^{-1} is a reasonable representation of all of the data.

We were unable to make rotational diffusion measurements on Xf at higher ionic strengths because of rapid deterioration of the samples. In 1 mM sodium phosphate the limited dependence of D_R for fd virus on virus concentration and ionic strength (Newman et al., 1977) has led us to surmise that the rotational diffusion coefficient obtained is, within its uncertainty limits, also valid for Xf in the higher ionic strength media used for translation diffusion measurements.

Translational Diffusion. Measurements on three virus samples at concentrations ranging from 37 to 146 $\mu g\ cm^{-3}$ gave the results listed in Table I. The data were analyzed by the method of cumulants, which give z-average translational diffusion coefficients (Koppel, 1972). Fitting the six values

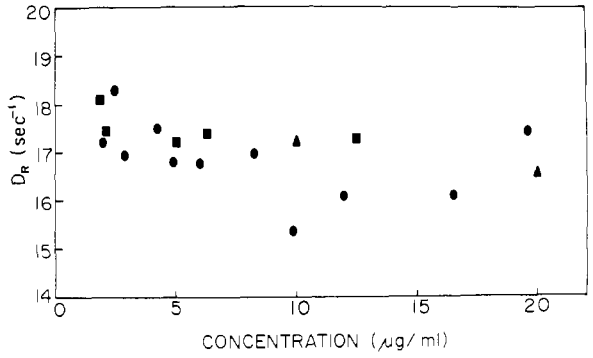

FIGURE 3: Rotational diffusion coefficients for Xf virus in 1 mM sodium phosphate, pH 7.5, corrected to 20 °C. The different symbols delineate results for different samples of monomers separated by agarose gel electrophoresis (■), by a single velocity centrifugation in a sucrose gradient (▲), and by two centrifugations in sucrose gradients (●).

to the relation $D_T = D_T^0 + (\partial D/\partial c)\Delta c$ gave $\partial D/\partial c = -(1.1 \pm 0.4) \times 10^{-5}\ g^{-1}\ cm\ s^{-1}$ and $D_T^0 = (2.58 \pm 0.03) \times 10^{-8}\ cm^2\ s^{-1}$. If there were, in fact, no concentration dependence, then $D_T = (2.49 \pm 0.03) \times 10^{-8}\ cm^2\ s^{-1}$. Our best estimate is $D_T^0 = (2.53 \pm 0.06) \times 10^{-8}\ cm^2\ s^{-1}$.

Dimensions of Xf Virus from the Broersma Equations. Broersma (1960a,b) established empirical relationships between the rotational and translational diffusion coefficients and length, L, and diameter, d, of a rigid rod. They are

$$D_R = [3kT/(\pi\eta L^3)](\delta - \zeta) \quad (3)$$

where $\delta = \ln \sigma$, with $\sigma = 2L/d$, and $\zeta = 1.45 - 7.5(1/\delta - 0.27)^2$ and

$$D_T = [kT/(3\pi\eta L)](\delta - \gamma) \quad (4)$$

where $\gamma = (\gamma_{\parallel} + \gamma_{\perp})/2 = -5.8(1/\delta)^2 + 4.15(1/\delta) - 0.017$. The numerical constants here differ slightly from Broersma's original relationships (Broersma, 1960a,b) through the inclusion of higher terms in calculations carried out more recently by Professor Broersma (private communication). The constants in eq 3 and 4 here were also used by Newman et al. (1977).

According to these relationships, L and d for the hydrodynamic species characterized by values of D_T and D_R are *uniquely* determined, as shown in Figure 4 with the data for Xf virus. The intersection of the two solid lines in Figure 4 gives the values of d and L which simultaneously satisfy both equations, and the intersections of the dashed lines give an indication of the uncertainties resulting from uncertainties in D_R and D_T. From this plot, we deduce a length of 980 ± 30 nm and a diameter of 7.2 ± 1.5 nm for Xf. As indicated in the legend to Figure 4 and under Materials and Methods, the value of D_R is for 20 °C but the measurements were done at temperatures between 5 and 12 °C in a low ionic stength buffer. All measurements of D_T were done at 25 °C in 0.15 M NaCl and 0.015 M sodium phosphate, pH 7. The dimensions obtained are thus subject to the assumption that the dimensions are the same under the two experimental conditions.

Discussion

The techniques of transient electric birefringence and intensity fluctuation spectroscopy are inherently accurate and straightforward, but the precision and accuracy of the results depend strongly upon the purity and homogeneity of the samples. Because of this, some workers have combined the separating technique with intensity correlation spectroscopy (Lim et al., 1977; Loh et al., 1979). The homogeneity of the sample can be determined to some extent from the diffusion

Table I: Translational Diffusion of Xf Virus in 0.15 M NaCl and 0.015 M Sodium Phosphate, pH 7, at 25 °C

sample	concn (μg cm^{-3})	diffusion coefficient, D_z (10^{-8} cm^{-2} s^{-1})
1	146	2.38
2	143	2.45
	73	2.50
	42	2.51
3	69	2.57
	37	2.52

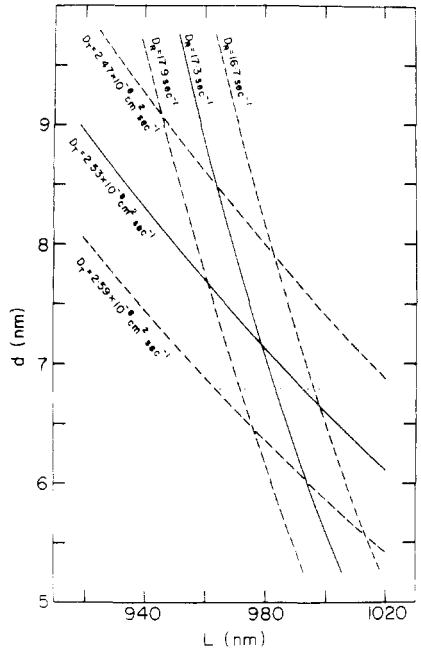

FIGURE 4: Plots of diameters and lengths of rigid rods satisfying eq 3 with $D_{R,20}$ = 17.3 ± 0.6 s^{-1} for η_{20} = 1.005 cP for 1 mM sodium phosphate and satisfying eq 4 with $D_{T,25}$ = (2.53 ± 0.06) × 10^{-8} cm^2 s^{-1} for η_{25} = 0.894 cP for 150 mM NaCl.

coefficient measurements themselves (Newman et al., 1977). The greater length heterogeneity and lower stability of Xf relative to fd made the collection of data in the present study more difficult than in the earlier study on fd (Newman et al., 1977) and restricted us to the lowest ionic strengths in the case of measurements of D_R. Nevertheless, we have carried out the full analysis using the Broersma relations to obtain dimensions from diffusion coefficients.

The hydrodynamic dimensions of 980 × 7 nm are in accord with the dimensions of 977 × 8 nm obtained under the conditions of electron microscopy (EM) (Kuo et al., 1969) and the diameter of 6 nm from X-ray diffraction (Marvin et al., 1974). The agreement between hydrodynamic length and EM length for Xf is similar to that observed earlier for fd (Newman et al., 1977) and seems to justify the application of the rigid rod model to these viruses in solution even though they appear to be flexible filaments according to electron microscopy. For such comparisons, it should be noted that the X-ray unit cell dimensions along the virus axis become shorter (<5%) as fibers are dried (Marvin et al., 1974; Dunker et al., 1974). Differences in hydrodynamic diameters of 7.2 ± 1.5 nm for Xf vs. 9.0 ± 1.0 nm for fd compare with differences in center-to-center distances for virions in fibers at 98% relative humidity measured by X-ray diffraction of 6.4 nm for Xf vs. 8.2 nm for fd. The hydrations one calculates for the structures in solution are 1.4 ± 0.5 cm^3 of solvent per g of fd virus (Newman et al., 1977) and 0.7 ± 0.3 cm^3 of solvent per g of Xf virus.

The length of Xf virus in solution multiplied by the mass per length of 19 100 daltons/nm (±10%) from turbidity measurements (Berkowitz & Day, 1980) gives a total virion molecular weight of 18.7 × 10^6 (±12%). This agrees with an independent value of 18.4 × 10^6 daltons (±5%) obtained by multiplying the number of protein subunits, 3700 (±5%), by 4963 daltons, the formula weight for the major coat protein subunit (4343 daltons) plus two nucleotides. The length of Xf in solution can be also used to calculate the axial translation per structure unit. Assuming that there are 3700 structure units and simply dividing the solution length by this number, we obtain an axial translation of 0.265 nm per structure unit. Dividing the apparent structure repeat of 7.7 nm in wet fibers from X-ray diffraction (Marvin et al., 1974) by the axial translation, we obtain 29 (±10%) structure units per apparent repeat. As the number of structure units per repeat is essential for proper interpretation of the X-ray patterns of Xf, but difficult to obtain from the patterns alone, we have calculated it here from independent physicochemical data.

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