Effect of coat protein mutations in bacteriophage fd studied by sedimentation analysis

Antonio D. Molina-Garcia,† Stephen E. Harding,† F. Guillermo Diaz,* Jose-Garcia de la Torre,*
David Rowitch, Richard N. Perham‡

University of Nottingham, Department of Applied Biochemistry & Food Science, Sutton Bonington LE12 5RD, United Kingdom; *Departamento de Quimica Fisica, Universidad de Murcia, Spain; and ‡Department of Biochemistry, University of Cambridge, CB2 1QW, United Kingdom

ABSTRACT (*a*) Bacteriophage fd is a filamentous virus that has previously been well characterized. (*b*) Earlier work using point mutagenesis indicated that a lysine residue at position 48 in the major coat protein plays a crucial role in interacting with the DNA and governing the assembly into an intact virion. (*c*) In this study the sedimentation properties (sedimentation velocity and equilibrium) of wild-type fd and two mutants substituted at lysine-48 (K48Q and K48A) were compared. (*d*) Both mutants are similar to each other [$M_r \simeq (19.5 \pm 1.5) \times 10^6$] but somewhat bigger than the wild-type [$M_r \simeq (15.1 \pm 1.5) \times 10^6$]. The value for the wild-type is consistent with earlier published values. (*e*) By combining these data with sedimentation coefficient data, it is possible to compare the contour lengths and relative flexibilities of the mutants with those of the wild-type virion. (*f*) The mutants are shown hydrodynamically to have larger contour lengths (as also observed by electron microscopy): the ~20% difference in values obtained assuming rigid particle hydrodynamics with those obtained from electron microscopy is strongly suggestive of some difference in flexibility between the wild-type and mutants.

INTRODUCTION

Bacteriophage fd is a class I filamentous and flexible phage whose molecular biology and structure have been well characterized (see, for example, Makowski, 1985; Webster and Lopez, 1985; Model and Russel, 1989). Bacteriophage fd particles are ~890 nm long and 7 nm in diameter, the cylindrical protein shell containing 2,700 molecules of the 50 amino-acid long major coat protein (Day and Wiseman, 1978) in a helical array (Marvin, 1978; Banner et al., 1981; Makowski and Caspar, 1981). This protein tube encapsulates a circular, positive-sense single-stranded DNA of 6,408 nucleotides which has been reported to account for 12% of the particle weight (Day and Wiseman, 1978).

Two selected point mutations in bacteriophage fd gene VIII, which encodes the major coat protein, were produced by site-directed mutagenesis (Hunter et al., 1987). These mutants caused a substitution of the lysine residue at position 48 in the wild-type protein by glutamine (fdg8Q48, referred to, in the rest of this study, as K48Q) or alanine (fdg8A48, referred to as K48A). Neither point mutation inhibited the assembly process and both mutant viruses, together with the wild-type fd phage, could be isolated and purified in sufficient quantity for biophysical analysis. Both mutant virions were ~35% longer than the filament of wild-type fd. The increase in particle length was attributed to a different mode of DNA packing in the mutants (Hunter et al., 1987), an explanation supported by further experiments that led to the construction of hybrid phage particles (Rowitch et al., 1988; Greenwood et al., 1991).

In addition to the change in length observed for the mutant virions, there were clear and characteristic differences in the electrophoretic mobilities of the K48A and K48Q mutants compared with wild-type fd when the particles were subjected to nondenaturing agarose gel electrophoresis. However, the mutations in the coat protein were on the inside of the virion, where the COOH-terminal domain of the protein abuts the DNA. Moreover, the changes in electrophoretic mobility of the virion did not correlate with the apparent change in charge on the coat protein, the K48Q virion migrating more slowly, and the K48A virion more rapidly than wild-type fd. Thus, it was suggested that the electrophoretic changes might be due to a subtle alteration of the conformation of the coat protein which could result in either a different superficial distribution of electrostatic charges or a significant alteration in particle flexibility (Hunter et al., 1987).

This study is an attempt to investigate these problems further by means of a more detailed physico-chemical approach, namely, estimation of (weight average) molecular weight, M_w (from sedimentation equilibrium), and translational conformational parameters (from sedimentation velocity analysis). From these data we estimate the length and relative flexibility of the K48Q and K48A mutants compared with native fd, using some recent theoretical treatments of the hydrodynamics of flexible particles (Garcia Molina et al., 1990).

MATERIALS AND METHODS

Bacteriophage fd

Wild-type bacteriophage fd was originally a gift of Dr. I. Molineux (Imperial Cancer Research Fund, London). The isolation and purifica-

A. D. Molina-Garcia's present address is Departamento de Quimica Fisica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain.

D. Rowitch's present address is The Children's Hospital, Boston, Massachusetts 02115.

Address correspondence to S. Harding.

Abbreviations used: K48A, fdg8A48 fd virus point amino-acid mutant; K48Q, fdg8Q48 fd virus point amino-acid mutant; QLS, quasi-elastic light scattering; M_r, molecular weight; M_w, weight-average molecular weight.

0006-3495/92/11/1293/06 $2.00

tion of the glutamine (K48Q) and alanine (K48A) mutants has been described previously (Hunter et al., 1987; Rowitch et al., 1988).

Solvents

Before hydrodynamic characterization, all samples were dialyzed extensively against a standard freshly prepared Na/K phosphate/chloride buffer (pH 6.5), made up to a combined ionic strength of 0.15 by adding the relevant proportions of NaCl according to Green (1933). (The solvent used here was similar to the one employed by Berkowitz and Day [1976] in an earlier study on wild-type fd).

Concentration measurements

Concentrations of bacteriophage fd solutions were determined spectrophotometrically using an LKB Ultrospec 4050 spectrophotometer controlled from a BBC microcomputer, or a Beckman Du-50 spectrophotometer connected to an Olivetti M24 personal computer, both with a 1-mm optical path length quartz cell. An extinction coefficient of 3.84×10^3 ml·g^{-1}·cm^{-1} at 269 nm (Berkowitz and Day, 1976) was used. This was also taken to be the same for the mutants. (In the worst case, i.e., where all the absorbance at 269 nm is assumed to be due to nucleic acid and not protein, the lower ratio of nucleic acid to protein in the mutants would lead to an underestimate of the virion concentration by ~30%. These errors would not affect the calculations for M_r from sedimentation equilibrium nor the $s^0_{20,w}$ values, but would influence estimates for the sedimentation concentration regression parameter, k_s). All concentrations for sedimentation velocity were corrected for radial dilution.

Sedimentation coefficient measurements

Sedimentation velocity experiments were performed using an MSE Centriscan analytical ultracentrifuge equipped with scanning absorption and Schlieren optics and a monochromator. Measurements were performed at a temperature of 20.0°C and at a speed of 16,000 rpm. Schlieren measurements were at a wavelength of 568 nm. Eight to twelve scans were used in determining each value of the sedimentation coefficient at a particular concentration (corrected for radial dilution). The sedimentation data were captured off-line using a Cherry digitizing Tablet interfaced to an Apple IIE computer which evaluated the sedimentation coefficient and the radial dilution correction factor for concentration.

All sedimentation coefficients, at finite concentrations, c, were corrected to standard conditions (water as solvent at 20°C) in the usual way:

$$s_{c(20,w)} = \frac{(1 - \bar{v}\rho)_{20,w}}{(1 - \bar{v}\rho)_{T,b}} \cdot \frac{\eta_{T,b}}{\eta_{20,w}} \cdot s_{T,b}, \qquad (1)$$

$\bar{v}$ being the partial specific volume, η the solvent viscosity, and ρ the solution density. A partial specific volume for bacteriophage fd particles of 0.720 ml/g (Newman et al., 1977) was employed. As with the extinction coefficient considered above, due to scarcity of material, this was taken to be the same for the mutants. (Differences in the weight fractions of nucleic acid between the mutants and the wild-type fd would lead to errors of ~1% in $\bar{v}$.) In all that follows the symbol s_c will be used in place of $s_{c(20,w)}$, and s corresponds to the value at infinite dilution. Infinite dilution values, s, ($s^0_{20,w}$) were obtained by fitting the (reciprocal) s_c data to

$$\frac{1}{s} = \frac{1}{s_c}(1 + k_s c), \qquad (2)$$

where k_s is the sedimentation concentration dependence regression coefficient. Use of solution densities for each concentration is rather difficult, tedious, and wasteful of material, and so we follow the common procedure of using solvent densities. This has no effect on the value, s, of s_c extrapolated to infinite dilution, and a simple correction exists for k_s (see, for example, Harding and Johnson, 1985).

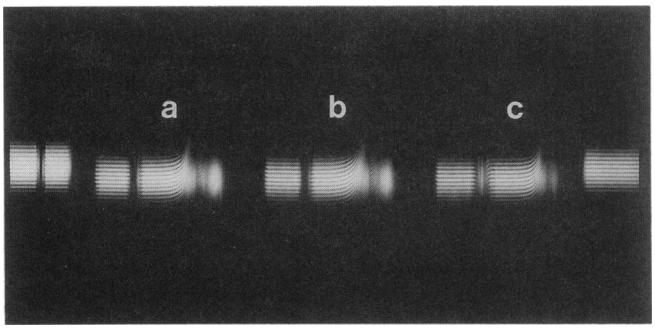

FIGURE 1 Rayleigh interference fringe profiles from native and mutant bacteriophage fd at sedimentation equilibrium. Rotor speed = 1,957 rev/min; temperature = 25.0°C. Initial loading concentration ~0.2 mg/ml. Solvent: standard phosphate chloride, pH = 6.5, I = 0.15. (a) native fd, (b) K48Q, (c) K48A.

Sedimentation equilibrium measurements

Sedimentation equilibrium experiments were performed using a Beckman Model E analytical ultracentrifuge equipped with Rayleigh interferometric optics and an RTIC temperature measuring system (set for 25.0°C). Samples were previously dialyzed for 24 h at 4°C and dialysis buffer was employed as reference. Although the procedure for the 'low' or 'intermediate' speed (Creeth and Harding, 1982) method was followed, because of the very high mass of fd particles, even at very low speeds (nominally 1,961 rpm), near-depletion conditions were obtained, with unavoidable loss of optical registration of the fringes near the cell base (Fig. 1). Nonetheless the meniscus concentration remained measurable and was obtained by mathematical manipulation of the fringe data (Creeth and Harding, 1982). The heavy 'rotor J' was used to minimize problems of instability at low speeds. Rayleigh interference patterns were recorded off-line using an adaptation (Harding and Rowe, 1988*a*, *b*) of an Ultrospec 2022 (LKB Instruments, Bromma) densitometer, and records of fringe displacement versus radial distance were obtained using the UCSD PASCAL routine ANALYSER (Harding and Rowe, 1988*a*, *b*).

Whole-cell apparent weight average relative molecular masses, $M_{w,app}$ were obtained by using the limiting value at the cell base of a directly determinable point average, "M^*" (Creeth and Harding, 1982); an independent estimate for the initial concentration was not required. To minimize the effects of thermodynamic nonideality, very low concentrations were employed (~0.2 mg/ml) in 30 mm optical path length cells (6 channel Yphantis type, with an inert oil to act as a 'false bottom' to the channels). Despite the low concentration used a small correction for nonideality (see, e.g., Tanford, 1961) was made using the formula $(1/M_w) \simeq (1/M_{w,app}) - 2Bc$, with $c \sim 0.2$ mg/ml. A value for the second virial coefficient B of 1.1×10^{-5} ml·mol·g^{-2} obtained earlier by Day et al. (1976) was used for wild-type fd: this value was taken approximately true also for the mutants, since for rigid rods of length L, over a limited range (BM/L) ≈ constant (see, e.g., Tanford, 1961).

RESULTS AND DISCUSSION

Homogeneity

Homogeneity was checked by sedimentation velocity. Single symmetrical Schlieren boundary peaks were ob-

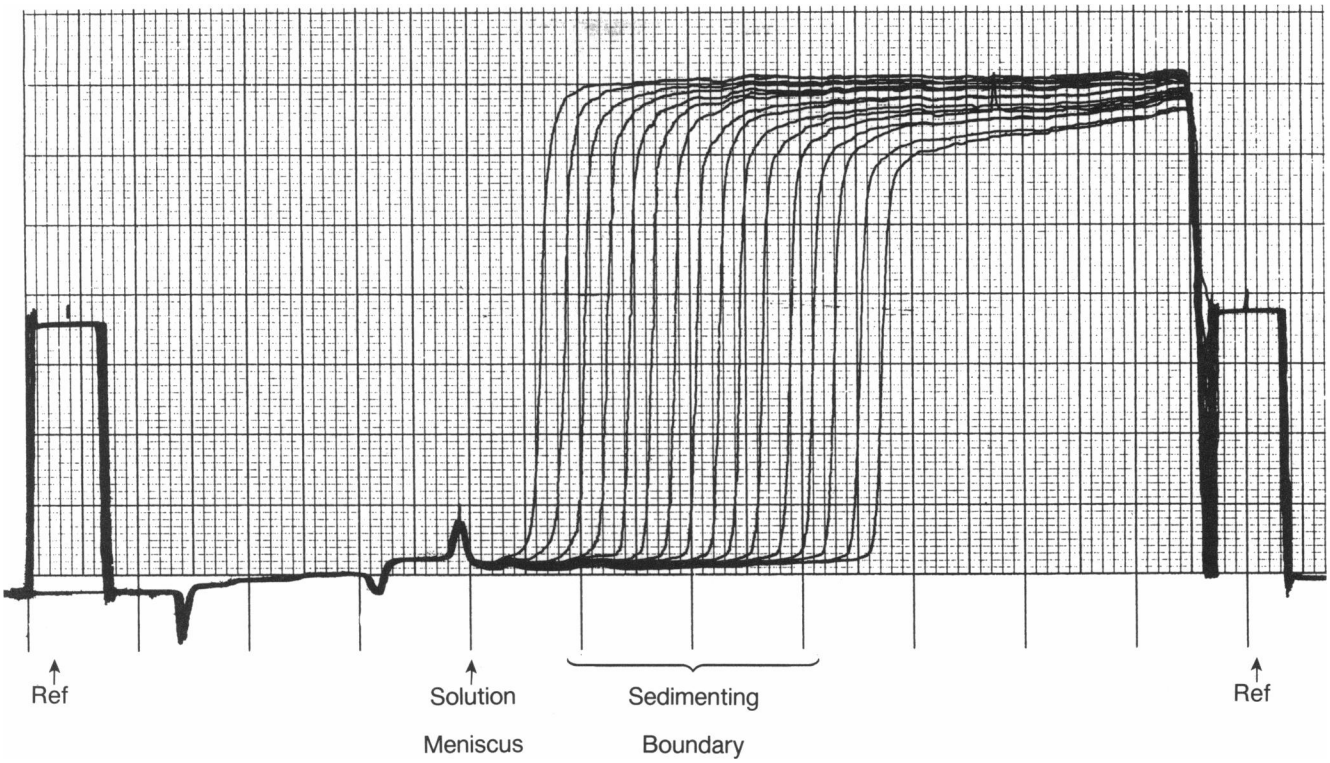

FIGURE 2 Sedimentation velocity diagram for a solution of bacteriophage fd (K48A mutant) obtained using the scanning Schlieren optical system on the MSE Centriscan. Rotor speed = 16,000 rev/min; temperature = 20.0°C. Loading concentration $c \sim 0.57$ mg/ml. The direction of sedimentation is from left to right. Scan interval = 9 min.

tained for all samples in sedimentation velocity experiments (Fig. 2) (although such symmetry is not in itself proof of homogeneity; see, for example, Gilbert and Gilbert, 1980). Schlieren peaks were 'hypersharp,' consistent with long rod-shaped particles with high exclusion volumes (Schachman, 1959).

Sedimentation coefficient measurements

Values for the sedimentation coefficients (corrected for water and 20°C and extrapolated to 'infinite dilution') are given in Table 1 for each of the variants studied. Four concentration points were employed for each determination. The dependence of the sedimentation coefficient on concentration (corrected for radial dilution effects) was represented by linear regression and produced a high degree of correlation in all cases (Fig. 3) Values for k'_s, the sedimentation coefficient concentration dependence regression factor (Table 1), were very high, as would be expected for very asymmetric particles with large thermodynamic exclusion volumes (Schachman, 1959) (although the precision with which this parameter can be measured, together with the assumptions concerning the extinction coefficient for the mutants does not allow a direct comparison between the three variants to be made).

The more precise sedimentation coefficient values also appear of the same order (Table 1), although within experimental error the mutants appear to sediment slower. The value of $s^0_{20,w} = (40.6 \pm 0.08) \times 10^{-13}$ s for wild-type fd is consistent with the value obtained earlier by Newman et al. (1977) for $s^0_{25,w}$ of $(47.0 \pm 0.3) \times 10^{-13}$ s (equivalent to an s [i.e., $s^0_{20,w}$] value of $(41.7 \pm 0.3) \times 10^{-13}$ s). However, there is no significant difference be-

TABLE 1 **Weight average molecular weight (M_w), (infinite dilution) sedimentation coefficient ($s^0_{20,w}$), concentration regression factor (k_s), and translational diffusion coefficient ($D^0_{t(20,w)}$) for native and mutant bacteriophage fd particles**

	$10^{-6} \times M_{w,app}$	$10^{-6} \times M_w$‡	$10^{-13} \times s^0_{20,w}$	k_s	$10^8 \times D^0_{t(20,w)}$
			s	ml/g	$cm^2\ s^{-1}$
fd (wild-type)	14.2 ± 1.5	15.1 ± 1.5	40.6 ± 0.8	292 ± 11	2.37 ± 0.29
K48Q	18.0 ± 1.5	19.5 ± 1.5	38.3 ± 0.6	272 ± 8	1.73 ± 0.17
K48A	18.0 ± 1.5	19.5 ± 1.5	36.4 ± 0.7	276 ± 9	1.65 ± 0.17

‡ After correction for nonideality.

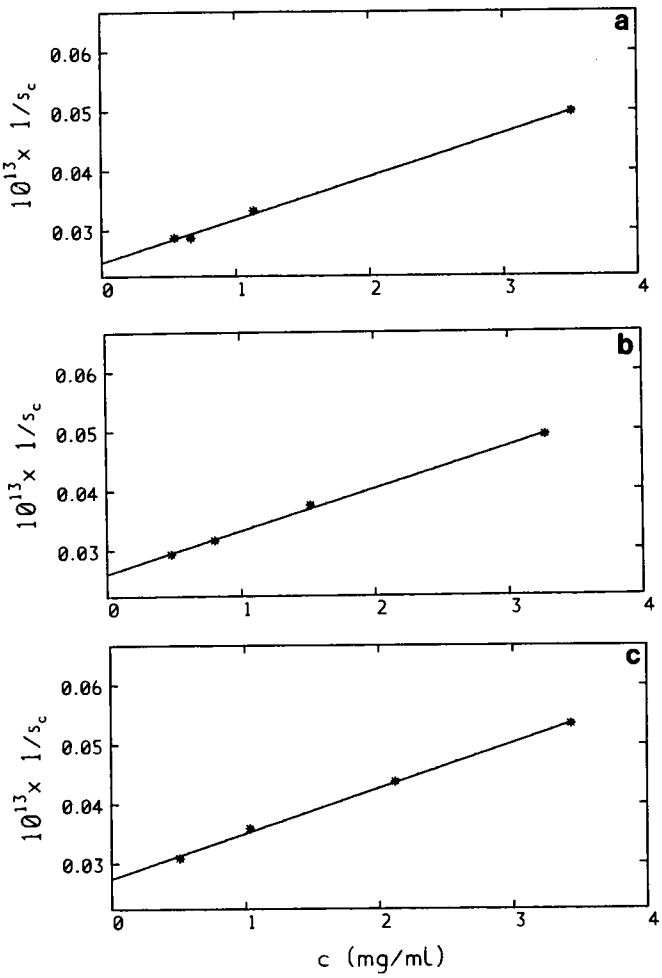

FIGURE 3 Plot of sedimentation coefficient vs. concentration for bacteriophage fd particles: (*a*) native, (*b*) K48Q, (*c*) K48A. Solvent: as in Fig. 1.

tween the s values of wild-type fd and the two mutants: this observation could be due either to genuine conformational similarities, or to part-cancellation of two opposing effects: increase in M_r and increase in asymmetry. Before any real conclusions with respect to conformation and flexibility can be drawn, therefore, it is important to establish the molecular weights of native fd and the mutants.

Molecular weight determinations

Low speed sedimentation equilibrium provides us with an unequivocal comparison of the molecular weights of fd, K48Q and K48A, without assumptions concerning conformation: the apparent weight average molecular weights (from the limiting value of the M^* function at the cell base (Fig. 4) as described above) and the corresponding "ideal" molecular weights (after the small correction for nonideality) are given in Table 1. Despite the larger errors expected from sedimentation equilibrium experiments for particles of this size (almost the top molecular weight limit) the differences can be clearly appreciated, with $M_w \sim 15 \times 10^6$ for fd and $M_w \sim 19.5 \times 10^6$ for both K48Q and K48A. The relatively large errors ($\sim \pm 10\%$) compared with the normal precision of the sedimentation equilibrium technique (usually better than $\pm 5\%$) reflects that we are on the upper molecular weight limits of applicability and the unavoidable feature of the extrapolations involved (cf. Fig. 4).

Our value for wild-type fd is consistent within experimental error with values given by Berkowitz and Day (1976) for the molecular weight using three independent techniques: sedimentation equilibrium ($\sim 14.5 \times 10^6$), classical light scattering coupled with electric birefringence ($\sim 14.3 \times 10^6$), and the DNA content coupled with the DNA molecular weight ($\sim 14.7 \times 10^6$). Newman et al. (1977) later gave a somewhat higher value $\{(16.4 \pm 0.6) \times 10^6\}$ calculated from the sedimentation coefficient (sedimentation velocity) and the translational diffusion coefficient as estimated by dynamic light scattering, although within experimental error this latter value is still consistent with our own estimate. It is still open to question, however, which of the previous values (viz those based $\sim 14.5 \times 10^6$ or the one based at $\sim 16.4 \times 10^6$) is the more reliable since no adequate explanation of the "lower value" from, e.g., sedimentation equilibrium has been offered (Newman et al., 1977) and particularly bearing in mind the difficulties encountered when applying dynamic light scattering to rod shaped virus particles (see, e.g., Johnson and Brown, 1992). For this study this is of no concern; we are simply interested in the relative differences between the wild-type fd and the two mutants, and indeed the value we use for the wild-type fd molecular weight is consistent with all the previous measurements.

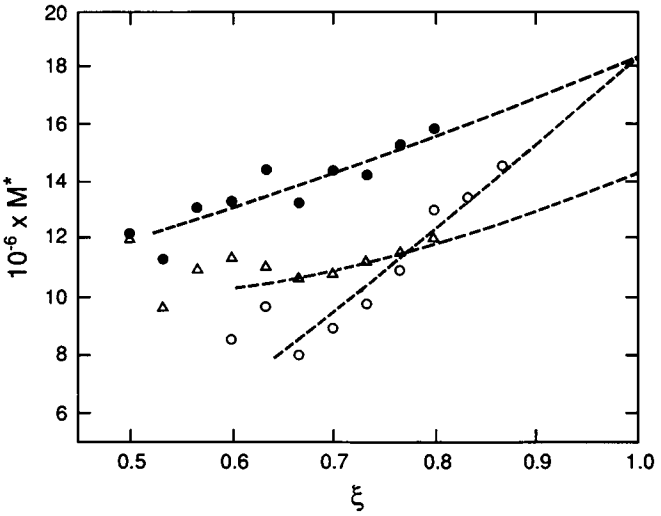

FIGURE 4 Plot of M^* vs. ξ for native and mutant fd bacteriophage particles at a loading concentration of ~0.2 mg/ml. Rotor speed = 1,960 rev/min; temperature = 25.0°C. Solvent: as in Fig. 1. $\xi = (r^2 - a^2)/(b^2 - a^2)$, r being the radial displacement, and a and b the corresponding values for the cell meniscus and base respectively. (△) Native fd; (●) K48A; (○) K48Q virus particles

In so far as the differences between wild-type and mutants are concerned, if the contour length of the K48A and K48Q mutants were increased by ~35% compared with wild-type fd, as indicated by electron microscopy (Hunter et al., 1987), and if the helical parameters of the coat protein assembly were essentially unchanged, the increased number of coat protein subunits (there is no change in DNA) would lead to an increase in the particle M_r. Given that the protein accounts for ~88% of the virion by weight (Day and Wiseman, 1978), the molecular weight should increase by ~30%, which accords well with the observed increase from $\sim 15 \times 10^6$ to $\sim 19.5 \times 10^6$ (Table 1).

Mathematical modeling

We are primarily interested in the length and relative flexibility of Q48 and A48 mutants compared with the wild-type fd. For the purposes of the comparison we make two assumptions: (*a*) that the wild-type can be represented hydrodynamically as a rigid rod, even though we know it to be somewhat flexible, as seen in the electron microscope; and (*b*) that the dimensions of wild-type fd estimated from electron microscopy are valid in solution: length, $L = (866 \pm 37)$ nm (Hunter et al., 1987); and diameter, $d = (8.0 \pm 0.4)$ nm (Newman et al., 1977, and references cited therein).

For ease of calculation it is more convenient to convert our s ($s^0_{20,w}$) values to equivalent values for the translational diffusion coefficient ($D^0_{t(20,w)}$) using the well known Svedberg equation (see, e.g., Tanford, 1961)

$$D_t = \frac{sRT}{M_r(1 - \bar{v}\rho_0)}. \quad (3)$$

These data are given in Table 1.

Although this is a rather indirect route to obtaining the translational diffusion coefficient, it avoids the problems of the more "direct" method (i.e., using quasi-elastic light scattering) since the strong asymmetry of the virus particles would require an extrapolation of the apparent diffusion coefficient (obtained at a finite angle) to zero angle (necessitating measurement at low angle, precisely where contamination problems through dust, etc., are their greatest [see, e.g., Godfrey et al., 1982]).

Wild-type fd

It is possible to compare our experimental value of $(2.37 \pm 0.29) \times 10^{-8}$ cm^2 s^{-1} for $D^0_{t(20,w)}$ with that which would be expected based on the assumptions above, viz., a rigid rod model with length and dimensions given by electron microscopy. For this we use the equation of Tirado and Garcia de la Torre (1979, 1980):

$$D_t = \frac{kT}{3\pi\eta_0 L}[\ln(L/d) + \gamma], \quad (4)$$

where k is the Boltzmann constant and γ is a small end-effect correction term. Using the limiting value, $\gamma \sim 0.3$ for a long rod ($L \to \infty$), Eq. 4 gives a value for $D^0_{t(20,w)} \sim 2.46 \times 10^{-8}$ cm^2 s^{-1}, in good agreement with the experimental data (this also corresponds to a mass per unit length, M_L, of $\sim 1.6 \times 10^4$ g mol^{-1} nm^{-1} and a volume, V, of 4.4×10^4 nm^3). Allowing for uniqueness problems (i.e., the possibility that other, nonrod models could fit the data equally well), this finding is at least consistent with the assumption that wild-type fd behaves hydrodynamically as a rigid rod in solution.

TABLE 2 **The effect of changes in rod contour length, *L*, and diameter, *d*, on the volume, *V*, and mass per unit length, M_L**

L	d	$10^{-4} \times V$	$10^{-4} \times M_L$
nm	*nm*	*nm^3*	*g·mol^{-1}·nm^{-1}*
900	35.8	91	2.16
1,000	26.7	56	1.95
1,100	19.7	34	1.77
1,200	14.5	20	1.63
1,250	12.4	15	1.56
1,300	10.5	11	1.50
1,400	7.6	6.4	1.39
1,450	6.5	4.8	1.34
1,500	5.5	3.6	1.30

All these pairs of (L, d) values reproduce a $D^0_{t(20,w)} = 1.69 \times 10^{-8}$ cm^2 s^{-1}.

Mutants

Q48 and A48 appear to have similar sedimentation properties. The weight average molecular weights are identical within experimental error and their sedimentation coefficients similar (Table 1).

The corresponding translational diffusion coefficients calculated from the Svedberg equation (Eq. 3 above) are $(1.73 \pm 0.17) \times 10^{-8}$ cm^2 s^{-1} for Q48 and $(1.65 \pm 0.17) \times 10^{-8}$ cm^2 s^{-1}. For the sake of the comparison with the wild-type fd, we will assume that both mutants have practically the same translational diffusion properties and take a mean value of 1.69×10^{-8} cm^2 s^{-1}. This value is appreciably lower than that of the wild-type. Differences in translational diffusion can be related to differences in dimensions (length and diameter), in flexibility, or in both aspects. An increase in dimensions of a rigid rod manifests as a decrease in the diffusion coefficient, while an increase in flexibility with fixed dimensions results in an increase of diffusivity. As D_t of the mutants is not larger, but instead smaller than that of the wild-type virus, flexibility alone cannot explain the difference.

However, it is clear that the observed decrease in D_t (from the wild-type virus to the mutant ones) can be due to an increase in size as reasoned as follows. If the mutants had the same density as the native form the volume should be $V_{(mut)} = V_{(wild\text{-}type)} \cdot [M_{r(mut)}/M_{r(wild\text{-}type)}] \sim 5.7 \times 10^4$ nm^3. Even with a large (~50%) tolerance in the permitted V, it is possible to show (Table 2), by comparing pairs of (L, d) values that reproduce $D_{t(20,w)} = 1.69 \times 10^{-8}$ cm^2 s^{-1}, that the criterion of equivalent V is accomplished in a small range of

lengths (1,350–1,500 nm). The best value is $L \sim 1400$ nm, for which V is only ~12% over that expected, and $M_L = 1.39$ is quite similar to that of the native form. Furthermore, the diameter is practically the same as that of the native form. This hypothesis would appear to lead, therefore, to the following conclusion:

The mutants are rod-like too, they have the same d and similar M_L as the native form, and the increase in M_r corresponds to an increase in length to ~1,400 nm. Results from electron microscopy also suggest a considerable increase in contour length for the mutants [to (1,153 ± 55) nm for Q48 and (1,178 ± 57) nm for A48] (Hunter et al., 1987). The overestimate of ~20% we obtain here based on rigid particle hydrodynamics (if experimentally significant) could be explained by different flexibilities of the mutants compared with the wild-type.

However, considerable caution has to be expressed when attempting to quantify flexibility based on sedimentation measurements alone: a useful criterion for flexibility is the ratio of the contour length, L, to the persistence length, a. If the flexibility parameter $L/a = 2$ (more than enough to be seen in electron microscopy) then the value of D_t would change only by ~5% (with $L/a = 5$ the difference would only be ~9%). This is to be compared with experimental errors of ~10% in $D^0_{t(20,w)}$ arising from errors in both $s^0_{20,w}$ and M_r.

Thus, although the experimental data for the wild-type and mutant viruses are compatible with straight rods, there could be an uncertain degree of flexibility in all of them that cannot be detected from measurements of $D^0_{t(20,w)}$ alone.

CONCLUSIONS

We have shown that the two point mutants (K48Q and K48A) of fd bacteriophage are hydrodynamically different from the wild-type bacteriophage. This difference can be due to an increase in the relative length of the mutant virions. In addition to the size change, some difference in flexibility cannot be ruled out, although translational diffusion measurements alone do not provide sufficient sensitivity to ascertain this. A model based on increased contour length of the mutants and possibly greater flexibility is consistent with the increased lengths of the mutant virions observed in the electron microscope (Hunter et al., 1987). To determine more precisely any contribution from changes in particle flexibility will require the incorporation of rotational hydrodynamic data.

The expert technical assistance of M.S. Ramzan (analytical ultracentrifugation) is greatly appreciated.

A. D. Molina-Garcia was supported by the European Social Fund. D. H. Rowitch was supported by the Oliver Gatty Fund.

Received for publication 28 October 1991 and in final form 22 June 1992.

REFERENCES

Banner, D. V., C. Nave, and D. A. Marvin. 1981. *Nature (Lond.)* 289:814–816.

Berkowitz, S. A., and L. A. Day. 1976. *J. Mol. Biol.* 102:531–547.

Bloomfield, V. A., D. M. Crothers, and I. Tinoco, Jr. 1974. Physical Chemistry of Nucleic Acids. Harper & Row Publishing Co., New York.

Creeth, J. M., and S. E. Harding. 1982. *J. Biochem. Biophys. Methods.* 7:25–34.

Creeth, J. M., and C. G. Knight. 1965. *Biochim. Biophys. Acta.* 102:549–558.

Day, L. A., and R. L. Wiseman. 1978. The Single-Stranded DNA Phages. D. T. Denhardt, D. Dressler, and D. S. Ray, editors. Cold Spring Harbor Laboratory, New York. 605–625.

Garcia Molina, J. J., and J. Garcia de la Torre. 1986. *J. Chem. Phys.* 84:4026–4030.

Garcia Molina, J. J., M. C. Lopez Martinez, and J. Garcia de la Torre. 1990. *Biopolymers.* In press.

Gilbert, G. A., and L. M. Gilbert. 1980. *J. Mol. Biol.* 144:405–408.

Godfrey, R. E., P. Johnson, and C. J. Stanley. 1982. Biomedical Applications of Laser Light Scattering. D. B. Sattelle, W. I. Lee, and B. R. Ware, editors. Elsevier, Amsterdam. 373–389.

Green, A. A. 1933. *J. Am. Chem. Soc.* 55:2331–2336.

Greenwood, J., G. J. K. Hunter, and R. N. Perham. 1991. *J. Mol. Biol.* 217:223–227.

Hagerman, P., and B. H. Zimm. 1981. *Biopolymers.* 20:1481–1502.

Harding, S. E., and P. Johnson. 1985. *Biochem. J.* 221:549–555.

Hunter, G. J., D. H. Rowitch, and R. N. Perham. 1987. *Nature (Lond.).* 327:252–254.

Johnson, P., and W. Brown. 1992. Laser Light Scattering in Biochemistry. S. E. Harding, D. B. Sattelle, and V. A. Bloomfield, editors. Royal Society of Chemistry, Cambridge. Chapter 11.

Koppel, D. E. 1972. *J. Chem. Phys.* 57:4814–4820.

Makowski, L., and D. L. D. Caspar. 1981. *J. Mol. Biol.* 145:611–617.

Makowski, L. 1985. Biological Macromolecules and Assemblies: Virus Structures. F. A. Jurnak, and A. McPherson, editors. John Wiley & Sons, New York. Vol. 1: 203–253.

Marvin, D. A. 1978. The Single-Stranded DNA Phages. D. T. Denhardt, D. Dressler, and D. S. Ray, editors. Cold Spring Harbor Laboratory, New York. 583–603.

Model, P., and M. Russel. 1988. The Bacteriophages. Vol. 2 R. Calendar, editor. Plenum Press, New York. 375–456.

Newman, J., H. L. Swinney, and L. A. Day. 1977. *J. Mol. Biol.* 116:593–606.

Rowe, A. J. 1977. *Biopolymers.* 16:2595–2611.

Rowitch, D. H., G. J. Hunter, and R. N. Perham. 1988. *J. Mol. Biol.* 204:663–674.

Sanders, A. H., and D. S. Cannel. 1980. Light Scattering in Liquids and Macromolecular Solutions. V. Degiorgio, M. Corti, and M. Giglio, editors. Plenum Press, New York. 173–182.

Schachman, H. K. 1959. Ultracentrifugation in Biochemistry. Academic Press, New York.

Tanford, D. 1961. Physical Chemistry of Macromolecules. John Wiley & Sons, New York. 380 pp.

Tirado, M. M., and J. Garcia de la Torre. 1979. *J. Chem. Phys.* 71:2581–2587.

Tirado, M. M., and J. Garcia de la Torre. 1980. *J. Chem. Phys.* 73:1986–1993.

Webster, R. E., and J. Lopez. 1985. Virus Structure and Assembly. S. Casjens, editor. Jones & Bartlett, Boston. 235–267.

Yamakawa, H. 1984. *Annu. Rev. Phys. Chem.* 35:23–47.