Hydrodynamic Properties and Molecular Weight of fd Bacteriophage DNA†

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ABSTRACT: We have measured the sedimentation coefficient, s, and the diffusion coefficient, D, for highly monodisperse samples of single-stranded circular DNA from the fd bacteriophage. The measurements of D were performed by intensity fluctuation spectroscopy, while s was determined by boundary sedimentation. For the two fairly high ionic strength solvents used in our experiments, SSC (0.15 M NaCl–0.015 M sodium citrate (pH 8)) and 1.891 M NaBr (pH 6.5), the DNA molecule forms a compact structure; therefore, the observed intensity fluctuation spectra (obtained with a digital autocorrelator) could be interpreted simply in terms of translational diffusion. The results of our measurements are, in SSC, $D_{20^\circ} = (6.63 \pm 0.18) \times 10^{-8}$ cm^2 sec^{-1} and $s_{20^\circ} = (24.6 \pm 0.4) \times 10^{-13}$ sec, and in 1.891 M NaBr, $D_{20^\circ} = (7.07 \pm 0.24) \times 10^{-8}$ cm^2 sec^{-1} and $s_{20^\circ} = (18.9 \pm 0.3) \times 10^{-13}$ sec. Combining the values obtained in SSC in the Svedberg equation, together with values for the density increment measured for fd DNA in SSC, yields $(1.87 \pm 0.06) \times 10^6$ for the molecular weight of fd NaDNA. The stated uncertainties represent 95% confidence limits. The molecular weight agrees within the experimental uncertainty with the values obtained by other absolute techniques reported in the preceding paper (S. A. Berkowitz and L. A. Day (1974), *Biochemistry 13,* 4825). Our measurements of the concentration dependence of D and s in SSC yield values for the corresponding virial coefficients which are in agreement with recent theoretical calculations of the virials for a hard sphere model, where the theoretical expressions are evaluated using the Stokes–Einstein radius (31.6 ± 0.6 nm) calculated from the diffusion coefficient. From a comparison of the dry and hydrated volumes of the equivalent hydrodynamic spheres for fd NaDNA, we calculate an effective hydration volume of 43 cm^3 of solvent per gram of DNA in SSC.

Hydrodynamic methods are widely used to characterize the size and overall shape of DNA molecules in solution. In addition to molecular weight determinations based on empirical relations such as those of Studier (1965) and Eigner and Doty (1965), intrinsic viscosity and sedimentation velocity data have been used to study changes in the shape of DNA as a function of solvent conditions and to establish the Kratky–Porod persistence length and solvation of DNA in solution (see, for example, Bloomfield *et al.*, 1974). Because of the large size and usual asymmetry of DNA, its diffusion has rarely been studied, although recently Strassburger and Reinert (1971) and Halsall and Schumaker (1972) have shown that reliable measurements of DNA diffusion coefficients can be performed by irreversible boundary spreading techniques.

The development of the technique of intensity fluctuation spectroscopy has made it possible to obtain the translational diffusion coefficient of macromolecules in solution at thermodynamic equilibrium with unprecedented accuracy in many systems (see, for example, Dubin *et al.*, 1970). Since the original demonstration of the principle of this method (Cummins *et al.*, 1964), the spectroscopic techniques have become vastly improved (see the volume edited by Cummins and Pike, 1974) and applied to many problems of biological interest (see Cummins, 1974, for a review).

The high absolute accuracy obtainable in intensity fluctuation spectroscopic measurements of the diffusion coefficient, together with corresponding accuracies obtainable in conventional sedimentation velocity measurements and in resonant oscillator densimetry, has led us to apply these methods to solutions of single-stranded fd DNA which were monodisperse with respect to molecular weight, and by choice of solvent, essentially monodisperse with respect to size and shape. These measurements yield a value for the molecular weight of fd DNA which has an absolute accuracy of 3.2% at the 95% confidence level. Our studies of the concentration dependence of the sedimentation and diffusion coefficients, together with the accompanying study of the thermodynamic properties of DNA samples from the same phage preparations (Berkowitz and Day, 1974), yield values for the sedimentation, diffusion, and thermodynamic virial coefficients. These virial coefficients for the polyelectrolyte molecule of fd DNA are accurately given by a hard sphere model which uses the experimentally determined Stokes–Einstein radius and involves no adjustable parameters.

Materials and Methods[1]

(*a*) *Preparation and Characterization of Samples.* The growth of fd bacteriophage and the isolation of its single-stranded DNA were described in the preceding paper. R17 bacteriophage, which was used in testing the spectroscopic system, was prepared following procedures described by Yamamoto *et al.* (1970) with the following modifications: no chloroform or lysozyme was used, two phase separations

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[1] For further details see Newman (1974) and Berkowitz (1974).

in polyethylene glycol were performed and, after digestions by 5 μg cm^{-3} pancreatic RNase and 25 μg cm^{-3} pronase, the samples were centrifuged to equilibrium in a CsCl density gradient. The R17 had a plating efficiency of 30% and was found to be monodisperse by analytical density gradient equilibrium ultracentrifugation and by gel electrophoresis.

Both the fd DNA and R17 solutions were brought to thermodynamic equilibrium of diffusible components by extensive dialysis at 4° against three changes of outer solution over a period of about 48 hr. The last outer dialysate solution was used for all dilutions and for base-line readings for absorption measurements. The solvents used in the diffusion studies were: ST (0.15 M NaCl–0.015 M Tris-HCl (pH 7.2)) for the R17 measurements; SSC (0.15 M NaCl–0.015 M sodium citrate (pH 8)) and 1.891 M NaBr (pH 6.5) for the fd DNA measurements. Solvents for the boundary sedimentation measurements were various dilutions of 10 X SSC (1.5 M NaCl–0.15 M sodium citrate (pH 8)) and 1.891 M NaBr. An Ostwald viscometer was used to measure, at 20.00 ± 0.01°, the viscosities of the SSC and NaBr solvents relative to water.

The two DNA preparations used were characterized for the fractions of linear and circular forms by band sedimentation velocity experiments under alkaline conditions, both before and after the diffusion measurements, as described in the preceding paper. The DNA in the samples used ranged from 82 to 97% circular with an average of 90%. The fraction of circular form present showed no discernible decrease after experiments using 400 mW of laser power. There was also no measurable change in the diffusion coefficients deduced from spectra obtained with the incident beam power ranging from a few milliwatts to 400 mW; the diffusion experiments were performed typically with only 50 mW.

Concentrations were determined by uv absorption measurements on Cary 14 recording spectrophotometers calibrated to ±0.5% absolute accuracy in absorbance with transmission filters. An extinction coefficient of $A_{1\,cm}(0.1\%) = 7.93$ mg^{-1} cm^2 at 260 nm was used for R17 in ST, which is an average of two literature values (Enger *et al.*, 1963; Gesteland and Boedtker, 1964), and a molar extinction coefficient $\epsilon(P) = 7370 \pm 110$ cm^{-1} M^{-1}, based on direct phosphorus determinations, was used for fd DNA in SSC (Berkowitz and Day, 1974).

Dust and other contaminants can seriously limit the accuracy of spectroscopic determinations of diffusion coefficients. We clarified the DNA samples by high speed centrifugation at 30,000*g* at 4° for about 2 hr in clear, thin-walled, sealed cellulose nitrate tubes (Beckman 302235 cut to hold 3 cm^3 of sample), which were used as the light scattering cells. A similar procedure was recently followed by Koppel (1974). This technique gave solutions which remained reasonably dust free for periods of 5 hr or more. The R17 samples were also cleaned by centrifugation. Concentration measurements were made directly on each sample after the correlation function measurements were taken. Measurements of the intensity of the scattered light as a function of height in the centrifuge tubes showed, within an uncertainty of about 2%, that no concentration gradient had been established during the clarification procedure.

(*b*) *Light Scattering Spectroscopic System.* The diffusion coefficient measurements were performed with an argon ion laser source (Spectra Physics 165) operating at either 488.0 or 514.5 nm. The laser beam was focused to a diameter of 0.1 mm in the sample cell which was mounted in the center of a thermostat bath constructed from an 11.5 cm diameter precision bore glass cylinder. The thermostat bath, which was centered on the axis of a goniometer, was controlled to ±0.01° at 20° by circulating filtered water from an external bath; the temperature was measured with an accuracy of 0.05°.

The collection optics and phototube were mounted on a counterbalanced arm which was free to rotate from 0 to 180° scattering angle with the pivot point at the center of the goniometer. Output pulses from a thermoelectrically cooled phototube (ITT F 4085) were amplified, standardized, and input to a 60 channel digital clipped correlator, designed by A. Fraser and built at the Johns Hopkins Applied Physics Laboratory. This correlator functions in a way similar to others described previously (Pusey *et al.*, 1974; Oliver, 1974).

(*c*) *Analysis of Spectroscopic Data.* The normalized clipped autocorrelation function of the photocurrent is given for a dilute monodisperse solution of spheres or small particles by (Jakeman and Pike, 1969; Oliver, 1974)

$$g_k^{(2)}(\tau) = 1 + A \exp(-2\tau/\tau_c) \qquad (1)$$

where k is the clipping level, τ_c is the correlation time of the optical field, and A is a parameter which depends on the number of coherence areas viewed, the clipping level, and deadtime effects. In our experiments we fit the measured correlation function to eq 1 with A and τ_c taken as adjustable parameters and the normalization determined independently from the total number of clipped and unclipped counts. The correlation time τ_c is related to the diffusion coefficient by

$$\tau_c^{-1} = Dq^2 \qquad (2)$$

where q is the magnitude of the scattering vector [$q = (4\pi/\lambda) \sin(\theta/2)$, where λ and θ are respectively the wavelength of light in the solvent and the scattering angle].

Measurements on the small spherical virus R17 (diameter $\simeq$ 27 nm) served as a test of the optical alignment and the general functioning of the spectroscopic system. We have found that the apparent diffusion coefficient, $(\tau_c q^2)^{-1}$, of a 0.83 mg cm^{-3} solution of R17 in ST at 20.0°C is independent of angle over a range of angles from 20 to 160°, with a root mean square deviation from the mean of 0.4%. For this concentration and solvent we find $D_{20} = 1.58 \times 10^{-7}$ cm^2 sec^{-1}, which yields $D_{20,w} = 1.60 \times 10^{-7}$ cm^2 sec^{-1}. This value for $D_{20,w}$ is 4% larger than the value reported by Pusey *et al.* (1972), as interpolated from a plot of the concentration dependence of $D_{20,w}$ for essentially the same sample conditions (see also Camerini-Otero *et al.*, 1974b).

The discrepancy between the two experiments is slightly greater than the combined uncertainty of our experiment (1.6%) and that reported by Pusey *et al.* (1%). In both experiments D was determined from least-squares fits of the logarithm of the normalized correlation function to first- and second-order polynomials in τ, and the values of D determined from the linear term of the second-order fits were about 1% greater than the first-order fit values (P. N. Pusey, personal communication). Pusey *et al.* reported the first-order value for D while we report second-order fit values because tests with computer-synthesized data designed to simulate the experimental problems of dust, stray light and aggregation indicate that when these problems are present, the error in D determined from a second-order

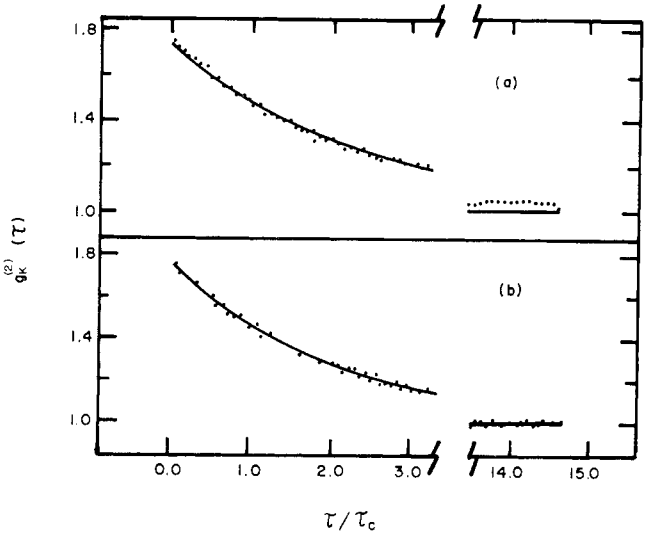

FIGURE 1: Intensity fluctuation spectra obtained at a 60° scattering angle for fd DNA in SSC at the lowest concentration measured (0.17 mg cm^{-3}). The data in the 16 delayed channels in the spectrum in (a), which was obtained for a sample containing a small amount of dust, are 1.9% higher than the calculated background, while in the spectrum in (b), obtained for a very clean sample, the observed and calculated levels in the delayed channels differ by only 0.04%. The diffusion coefficient determined from a second-order fit to the spectrum in (a) (with the calculated value for the background) is 3% lower than the diffusion coefficient determined from the spectrum in (b).

analysis is approximately half as large as that from a linear analysis. Our stated uncertainties include, in addition to the statistical uncertainty, a 1% uncertainty from the fitting procedure.

Another possible source of the difference between the two experiments is the difference in the procedures followed in preparing the R17 (see Camerini-Otero *et al.*, 1974b). Finally, we note that if the value of $D_{20,w}°$, deduced from our data and the virial coefficient of Pusey *et al.*, is combined with the values of $s_{20,w}°$ and partial specific volume used by Pusey *et al.*, then the resultant value for the molecular weight, 3.86×10^6, is only 0.7% larger than the average of measurements by other techniques (Camerini-Otero *et al.*, 1974a).

One feature of our correlator which is particularly useful in checking for the presence of dust in the samples is a delay of 128 channel times between channels 44 and 45 of our 60 channel correlator. Typically, data were collected in such a way that 1.8 correlation times of the measured spectrum were spanned in the first 44 channels so that the last 16 channels should, for data described by a single exponential, be within the noise of the background. Each correlation function measurement was subjected to a background check with the use of the delay line as illustrated in Figure 1. Two spectra are shown, one having data significantly above the background level expected for the delayed channels and one having data in good agreement with the expected background level. Dust particles in the scattering volume produce the higher background level. For all the data reported here, there was agreement between the measured and calculated background within the uncertainty in the background ($\simeq$0.1%). As a further justification for fitting the data to a single exponential, several spectra were taken spanning different numbers of correlation times. For the same experimental conditions, the same result for τ_c was obtained within 1% when the first 44 channels spanned anywhere from 1 to 10 correlation times.

(*d*) *Velocity Sedimentation.* A Beckman Model E analytical ultracentrifuge, equipped as described in the preceding paper, was used for the sedimentation experiments. Runs were made at 40,000 rpm ($\sim$120,000*g*) with accurate values for the speeds determined with a revolution counter and stopwatch. The temperature of the rotor was adjusted in the chamber to slightly above 20° before acceleration. Once final speed was attained, deviations from 20° were corrected with either the refrigerator or the heating unit and then left uncontrolled to avoid convection. In most runs the temperature remained at 20.0 ± 0.2° without further adjustment, but in a few the deviation exceeded 0.2° and one additional adjustment was necessary. The photoelectric scanner was used to record the position of the sedimenting boundary, which was taken as the intercept of the trace with the half-height of the plateau since all boundaries were very symmetrical and sharp. Sedimentation coefficients were obtained from linear least-squares analyses of the logarithm of the radial distance of the boundary *vs.* time and thus represent values corresponding to the average concentration measured in the plateau region between the first and last scans.

Results

The results are summarized in Table I, which also includes the density of the solvents used in the diffusion studies and the density increment of the DNA in these solvents. The errors given in Table I are the uncertainties obtained from a Student *t* test and, where appropriate, from our best estimates of any systematic errors, both at the 95% confidence limit.

(*a*) *Diffusion Coefficients.* Spectra were collected for fd DNA at four or six angles from 35 to 145°, with about eight spectra (typically 200-sec experiments) taken at each angle. The diffusion coefficients [$D = (\tau_c q^2)^{-1}$] deduced from fitting the spectra to eq 1 showed no angular dependence for the DNA samples in either SSC or 1.891 M NaBr; the root mean square deviation in the values of D determined from a series of measurements as a function of angle was typically 0.3%. The values for the diffusion coefficient at a given concentration were therefore obtained from weighted averages of the values measured at all angles.

Measurements were performed at a nominal temperature of 20° on two preparations of DNA obtained from different bacteriophage preparations, and temperature corrections to the viscosity, which were at most 0.5%, were applied when necessary to find D_{20}. In Figure 2 the results for D_{20} in SSC are plotted as a function of concentration from 0.18 to 1.8 mg cm^{-3}. Similar results were obtained from measurements in 1.891 M NaBr although the concentration dependence was not as extensively investigated.

The diffusion coefficient can be written (see, *e.g.*, Tanford, 1961)

$$D = (c/N_a f)(\partial \mu/\partial c) \qquad (3)$$

where c, N_a, f, and μ are, respectively, the concentration, Avogadro's number, the frictional coefficient, and the chemical potential. Expanding μ and f in power series in the concentration, we have $(\partial\mu/\partial c) = (N_a kT/c)(1 + 2BMc + \ldots)$ and $f = f°(1 + B'c + \ldots)$; hence

$$D = D°[1 + (2BM - B')c + \ldots] \qquad (4)$$

where $D° \equiv kT/f°$. The data were analyzed to first order in the concentration for both solvents since the standard F test showed that there was no significant improvement

TABLE I: Summary of Our Measurements of the Properties of fd DNA.[a]

Property	Solvent	
	SSC	1.891 M NaBr
Solvent refractive index[b]	1.334	1.364 ± 0.002
Solvent/water viscosity ratio[c]	1.026 ± 0.008	1.142 ± 0.010
Solvent density (g cm^{-3})[c]	1.0060 ± 0.0002	
Density increment, $(\partial\rho/\partial c)_\mu$[d]	0.483 ± 0.010	
Diffusion coefficient, D_{20}° (10^{-8} cm^2 sec^{-1})	6.63 ± 0.18	7.07 ± 0.24
Sedimentation coefficient, s_{20}° (10^{-13} sec)	24.6 ± 0.4	18.9 ± 0.3
Molecular weight, M (10^6 g mol^{-1})[e]	1.87 ± 0.06	
Number of nucleotides[e]	5650 ± 180	
Radius of gyration, R_G (nm)[d]	41.6 ± 3.5	
Debye–Hückel screening length, R_{DH} (nm)	0.62	0.22
Hydrodynamic radius, R_h (nm)	31.6 ± 0.6	26.6 ± 0.7
Volume of hydration, V_h (cm^3/g of DNA)[f]	43 ± 8	
Sedimentation virial, B' (g^{-1} cm^3)	287 ± 28	
Thermodynamic virial, $2BM$ (g^{-1} cm^3)[d]	320 ± 170	
Diffusion virial, $2BM - B'$ (g^{-1} cm^3)	51 ± 16	40 ± 90

[a] Errors quoted are for 95% confidence limits, including systematic as well as statistical uncertainties. [b] At 488 nm and 20°; SSC value from handbook, 1.891 M NaBr value measured with an Abbé refractometer. [c] At 20°. [d] From Berkowitz and Day (1974). [e] Na salt of single-stranded fd DNA; the error includes 1.4% uncertainty (at the 95% confidence limit) in the concentrations and average nucleotide molecular weight. [f] See Discussion for the method of calculation; the error includes 10% uncertainty from the use of ϕ' in place of $\bar{v}$.

when a quadratic term was included. The results for D_{20}° and $(2BM - B')$, obtained by a weighted linear least-squares analysis, in both SSC and 1.891 M NaBr, are given in Table I along with the Debye–Hückel screening length, R_{DH}, and the value of the radius, R_h, of the equivalent hydrodynamic sphere based on the Stokes–Einstein relation, $D = kT/6\pi\eta R_h$ (where η is the solvent viscosity).

(*b*) *Sedimentation coefficients* of fd DNA in SSC and 1.891 M NaBr solvents were determined for solutions ranging from 0.003 to 2.125 mg cm^{-3} and 0.007 to 0.09 mg cm^{-3}, respectively, and the results in SSC are shown in Figure 2.

Since s is proportional to f^{-1}, the concentration dependence of s is given by

$$s = s^\circ(1 + B'c + \ldots)^{-1} \quad (5)$$

Our results for s° and B' were obtained from a weighted linear least-squares analysis of the data with the quadratic and higher order terms in eq 5 neglected (see Figure 2); a fit to eq 5 with the quadratic term included showed no improvement in the fit, as determined using an F test.

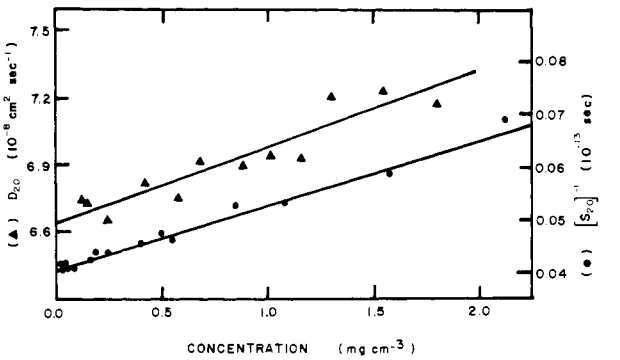

FIGURE 2: The concentration dependence of D_{20} (▲) and s_{20} (●) for fd DNA in SSC.

Sedimentation coefficients were also measured for DNA in different dilutions of 10 X SCC over a concentration range of 0.003–0.04 mg cm^{-3}. The relative compactness of fd DNA in a series of dilutions of 10 X SSC can be seen in Figure 3 in which $s_{20,w}^\circ$ values obtained for these solvents are plotted as a function of total ionic strength; also shown in Figure 3 is the molar extinction coefficient at 259 nm, ϵ(P), which is sometimes used as an indicator of secondary structure. The hydrodynamic radius in SSC is almost minimal, as Figure 3 illustrates. Experimental differences in the sedimentation rate of linear and circular molecules in neutral aqueous solutions were seen only in 0.1 X SSC (ionic strength $\simeq$ 0.024 M).

In order to compare the results of our sedimentation coefficient measurements with the results of previous experiments (see Discussion), values for the coefficients in water, s_w°, were converted to values in the solvents, s°, using the relation

$$s^\circ = s_w^\circ(\eta_w/\eta)[(\partial\rho/\partial c)/(\partial\rho/\partial c)_w] \quad (6)$$

(*c*) *Molecular Weight and Hydration.* We calculate a value for the molecular weight of the sodium salt of fd DNA (given in Table I) by combining our measured values for D_{20}° and s_{20}° with a value for $(\partial\rho/\partial c)_\mu$ (Berkowitz and Day, 1974), obtained for the DNA in SSC, in the Svedberg equation

$$M = \frac{s^\circ RT}{D^\circ(\partial\rho/\partial c)_\mu} \quad (7)$$

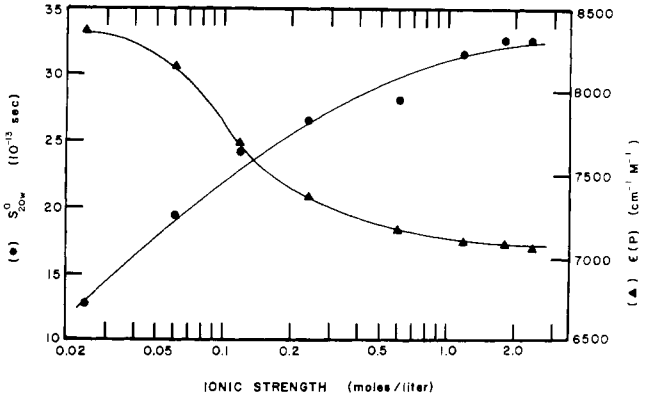

FIGURE 3: The dependence of $s_{20,w}^\circ$ (●) and the molar extinction coefficient at 259 nm, ϵ(P) (▲), on the total ionic strength of the solvent for fd DNA in a series of dilutions of 10 X SSC. The smooth curves are drawn to guide the eye. The ionic strength of SSC is 0.24 mol/l.

where R is the molar gas constant and $(\partial\rho/\partial c)_\mu$ is the density increment, which is related to the apparent specific volume ϕ', extrapolated to zero concentration, by $(\partial\rho/\partial c)_\mu \equiv 1 - \phi'\rho$ (Casassa and Eisenberg, 1964).

The quantity directly determined by the sedimentation and diffusion coefficients is the buoyant molecular weight, $M(\partial\rho/\partial c)_\mu$. Since $(\Delta\rho)$ is directly measured, the molecular weight calculated from the buoyant molecular weight is that for the species for which the weight concentration is defined (Casassa and Eisenberg, 1961). Ultimately, all our concentration measurements are based on the conversion from phosphorus concentration, and since we chose to convert this to the concentration of NaDNA (one molecule of Na per nucleotide), the molecular weight we have found is that of NaDNA.

Our measured values for D_{20}° are the z average values, while we have determined the weight-average values of s_{20}°. Kinell (1959) and Koppel (1972) have shown that if the weight-average s_{20}° and z average D_{20}° are used in the Svedberg equation, then the weight-average molecular weight is obtained directly, and it is this value that is given in Table I. Also given in the table are the number of nucleotides per DNA molecule, based on 331 daltons/nucleotide (Marvin and Hoffmann-Berling, 1963b; Schaller *et al.*, 1969; Wiseman *et al.*, 1972) and the effective volume of hydration per gram of dry DNA, assuming a spherical shape for the hydrodynamic particle (see Discussion).

Discussion

(*a*) *Monodispersity.* A possible source of uncertainty in the diffusion measurements is the degree of polydispersity of the DNA samples. Three sources of polydispersity can be distinguished: (1) contamination and fragments or aggregates of the DNA, (2) a single nick in the circular DNA leading to a linear species with the same molecular weight, and (3) configurational differences of the circular molecules (Forsheit and Ray, 1970). We conclude that polydispersity from fragments or aggregates of the DNA and configurational polydispersity are not significant since, within the detectability limit of the sedimentation measurements, no other sedimenting species was observed. We also conclude that for our samples the polydispersity from nicking has a negligible effect on D, since independent tests indicate a high degree of intactness and, moreover, at the high ionic strength used in these experiments, singly nicked DNA sediments at the same rate as intact DNA.

A second-order analysis of the diffusion data provides a direct measure of polydispersity since the normalized coefficient of the quadratic term, $\sigma_D/2$, is the z average normalized variance of the distribution of D values. For a random coil molecule, assuming that $D \propto M^{-\alpha}$ and $[(\langle M_z\rangle/\langle M_w\rangle) - 1] < 0.25$, Pusey (1973, 1974) has shown that $\sigma_D = \alpha^2[(\langle M_z\rangle/\langle M_w\rangle) - 1]$, where $\langle M_z\rangle$ and $\langle M_w\rangle$ are the z and weight-average molecular weights. Assuming a value for $\alpha = 0.6$, based on data for native linear DNA (Reinert *et al.*, 1971), we find that for our DNA samples in SSC and NaBr $\langle M_z\rangle/\langle M_w\rangle = 1.049$ and 1.025, respectively. The interpretation of σ_D in terms of molecular weight polydispersity indicates that our samples are highly monodisperse; moreover, σ_D arises from other sources of polydispersity as well as molecular weight polydispersity. Thus, the above values for $\langle M_z\rangle/\langle M_w\rangle$ represent an upper limit for the molecular weight polydispersity, and in fact the ratio obtained by equilibrium ultracentrifugation of DNA samples in SSC prepared in the same way as for the diffusion studies is smaller, $\langle M_z\rangle/\langle M_w\rangle = 0.99 \pm 0.06$ (Berkowitz and Day, 1974).

(*b*) *Previous Studies.* Wiseman obtained a value of 24.8×10^{-13} sec for $s_{20,SSC}^\circ$ for fd DNA in SSC, which is 0.9% higher than our value for $s_{20,SSC}^\circ$ (R. L. Wiseman, personal communication; also see Wiseman *et al.*, 1972). An earlier measurement of the sedimentation coefficient of fd DNA in a solvent similar to SSC by Marvin and Hoffmann-Berling (1963a,b) gave a result about 5% smaller than our value.

Halsall and Schumaker (1972) used ultracentrifugation techniques to determine both the sedimentation and diffusion coefficients of M13 bacteriophage DNA, which is virtually identical with fd DNA. Their measurements were performed in 1.891 M NaBr, but the reported coefficients were the hypothetical values in water, $s_{25,w}^\circ$ and $D_{25,w}^\circ$. Converting these coefficients to the NaBr solvent at 20° using handbook values for the viscosity and density of 1.891 M NaBr and using their assumed value of ϕ', 0.556 g^{-1} cm^3, we obtain $s_{20,NaBr}^\circ = 18.3 \times 10^{-13}$ sec and $D_{20,NaBr}^\circ = 7.4 \times 10^{-8}$ cm^2 sec^{-1}. These values are 3 and 5% lower, respectively, than our values for the corresponding coefficients, although the differences between the coefficients obtained in the two experiments are within the combined reported uncertainties. Lacking a value for $(\partial\rho/\partial c)_\mu$ for M13 DNA, Halsall and Schumaker calculated, from their values for $s_{25,w}^\circ$ and $D_{25,w}^\circ$, a value for the buoyant molecular weight, $M(1 - \phi'\rho)_{25,w} = 7.41 \times 10^5$. This value, however, is not independent of the assumed value for ϕ', as Halsall and Schumaker appear to assert, since in correcting s to a hypothetical value in water, a value for ϕ' was assumed. If we calculate a buoyant molecular weight based on their values for s and D in 1.891 M NaBr, we find $M(1 - \phi'\rho)_{20,NaBr} = 6.02 \times 10^5$, which is 8.2% lower than our value, 6.56×10^5.

The agreement of the Halsall and Schumaker diffusion coefficients with our measurements indicates that reasonably accurate determinations of D can be obtained using an ultracentrifuge, which is a more common biological laboratory instrument than a digital correlator. It should also be noted that the technique of Halsall and Schumaker can, in principle, be applied to solutions of mixtures of macromolecules when a specific assay exists for the molecule under study (Halsall and Schumaker, 1972).

(*c*) *Concentration Dependence.* Circular, single-stranded fd DNA is a polyelectrolyte molecule, the interparticle interactions of which would appear to be quite complicated. However, we have found that the concentration dependence for both s and D can be interpreted simply in terms of an equivalent uncharged rigid sphere model with a radius equal to the Stokes–Einstein hydrodynamic radius.

There have been several calculations of the concentration dependence of s for uncharged hard spheres, all of which can be expressed as

$$s = s^\circ(1 - K_s\phi + \ldots) \qquad (8)$$

where $\phi = (4\pi/3)r^3cN_a/M$ is the volume fraction of the solute (r is the sphere radius) and the (dimensionless) virial coefficient, K_s, is related to B' in eq 5 by $K_s = B'c/\phi$. Burgers (1941,1942) calculated $K_s = 6.587$, and Pyun and Fixman (1964) subsequently refined this calculation and obtained $K_s = 7.157$. Recently, Batchelor (1972) has reconsidered the problem and obtained $K_s = 6.55$, pointing out several deficiencies in the previous two calculations. If we interpret our sedimentation data in SSC in terms of the

TABLE II: Dimensionless Virial Coefficients.

Virial	Hard Sphere Model	Expt
K_s (sedimentation)	6.55[a]	6.7 ± 0.8
ζ (thermodynamic)	8	7.9 ± 2.6[b]
		7.6 ± 3.9[c]
$\zeta - K_s$ (diffusion)	1.45[d]	1.2 ± 0.4
	1.3–1.9[e]	

[a] Batchelor (1972). [b] Deduced from measured values for K_s and $\zeta - K_s$. [c] From equilibrium sedimentation (Berkowitz and Day, 1974). [d] From Batchelor's value of K_s. [e] Altenberger and Deutch (1973), for the range of angles studied in our experiments.

hard sphere model with sphere radius $r = R_h$, we find K_s = 6.7 ± 0.8, in agreement with the theoretical value (see Table II).

The sedimentation virial coefficient predicted by the hard sphere model can be combined with the thermodynamic virial coefficient for this model to yield a value for the diffusion virial coefficient. For this purpose the thermodynamic virial coefficient can be written in dimensionless form, $\zeta = 2BMc/\phi$. The thermodynamic virial coefficient is related to the excluded volume per particle, V_e, by $2BM = N_a V_e/M$; hence for hard spheres the dimensionless thermodynamic virial is given by $\zeta = 8$. Direct measurements of the thermodynamic virial coefficient (over the same concentration range studied in diffusion and sedimentation experiments) using equilibrium sedimentation techniques were made by Berkowitz and Day (1974). Their result for $2BM$ for fd DNA in SSC, given in Table I, when converted to the dimensionless parameter ζ, is 7.6 ± 3.9, in agreement with the hard sphere model value.

Combining the hard sphere model values for the thermodynamic and sedimentation coefficient virials yields for the dimensionless diffusion coefficient virial, $\zeta - K_s = 1.45$; our measured value, 1.2 ± 0.4, is in agreement with this theoretical prediction. Our value for $\zeta - K_s$ can also be compared directly with the recent theoretical predictions of Altenberger and Deutch (1973), who calculated the concentration dependence of D for rigid spheres. Their result predicts that, for spheres with a diameter of 63.2 nm, the apparent value of D, $(\tau_c q^2)^{-1}$, should decrease by 5.6% at our highest concentration as the angle is varied from 35 to 145° and that the virial $\zeta - K_s$ should vary between 1.28 and 1.92 as the angle is decreased within the same range, leading to a range of values for K_s from 6.1 to 6.7. Our value of $\zeta - K_s$ is in the range of the Altenberger and Deutch predictions; however, we observed no variation of the apparent diffusion coefficient with angle within 1% at any concentration.

Table II provides a comparison of the virial coefficients which were directly measured with those calculated for the hard sphere model. Note that any two of the directly measured values for ζ, K_s, and $\zeta - K_s$ can be combined to obtain the third virial coefficient, as we have done, for example, for ζ in Table II. The comparison between theory and experiment involves the conversion from weight concentration to volume fraction of DNA by using experimental values for M and R_h. The agreement between the hard sphere model predictions and our measurements is, thus, all the more striking since each experimental value for a dimensionless virial coefficient is actually determined from three or four independently measured quantities.

Our measurements indicate that at an ionic strength of 0.24 M, that of SSC, even at the highest concentrations measured, there are no strong electrostatic effects between DNA molecules. This is reasonable if one considers that the Debye–Hückel radius is about 0.62 nm at this ionic strength and that the average interparticle spacing is about 100 nm even at a concentration of 1.8 mg cm^{-3}.

(*d*) *Solvation.* The value for the effective water of hydration given in Table I was obtained by comparing the hydrodynamic volume of the equivalent Stokes–Einstein sphere to the volume of the DNA based on a density of $(\phi')^{-1}$. Lacking a value for the partial specific volume of the dry DNA, $\bar{\nu}$, which would enable us to calculate the volume of the dry DNA, we have used our measured value of ϕ' in place of $\bar{\nu}$. Based on the data for double-stranded DNA obtained by Cohen and Eisenberg (1968), who found that in 0.15 M NaCl $\bar{\nu}$ was about 8% less than ϕ', we expect the error in using ϕ' in place of $\bar{\nu}$ in calculating the solvation to be less than 10%. The solvation value reported here represents the hydrodynamically trapped solvent per gram of DNA within the Stokes–Einstein volume associated with each DNA molecule, and not solvent molecules bound to the DNA (see, for example, Tunis and Hearst, 1968). Thus, as the ionic strength of the solvent is varied, the effective hydration can change considerably; for example, from our values for $(\partial\rho/\partial c)_\mu$ and s° at 0.01 X SSC and from our value for D at 1.891 M NaBr, we calculate that in these two solvents there are, respectively, about 300 and 26 cm^3 of trapped solvent per gram of DNA.

(*e*) *Intrinsic Viscosity.* The semiempirical relation of Mandelkern and Flory (1952)

$$M = \left[\frac{s_{20}{}^0[\eta]^{1/3}\eta N_a}{\beta(\partial\rho/\partial c)_\mu}\right]^{3/2} \quad (9)$$

where $[\eta]$ is the intrinsic viscosity of the DNA in g^{-1} dl and $s_{20}{}^\circ$ is in Svedbergs, has been shown to describe the molecular weight of native and denatured DNA over a wide range of molecular weights with β = 2.3–2.5 × 10^6 (Eigner and Doty, 1965; Aten and Cohen, 1965; Rosenberg and Studier, 1969). By substituting our measured values for all the other quantities in the relation, we can obtain a value for $[\eta]$ in SSC of 1.37–1.76 g^{-1} dl based on the range of β given. Our calculated value is in agreement with the value of $[\eta]$ = 1.5 g^{-1} dl obtained by Marvin and Hoffmann-Berling (1963b) for fd DNA in a similar solvent to SSC.

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