Optical and Hydrodynamic Studies of the Structure of Bacteriophage f2†

Robert W. Henkens* and John L. Middlebrook

ABSTRACT: Circular dichroism, combined with hydrodynamic measurements of gross size and shape, has been used to study the structure of the protein and nucleic acid components of bacteriophage f2. Short alkaline treatment of this virus results in liberation of the RNA, without major alteration of the circular dichroism, leaving a capsid which behaves hydrodynamically as a solvent-filled protein shell. The combined circular dichrosim spectra of the isolated protein shell and RNA components is very similar to the circular dichroism of intact f2. These results indicate that protein–nucleate interactions in the virus structure do not result in major conformational alteration of the macromolecular components. The far-ultraviolet circular dichroism of the capsid, which has a single minimum near 217 nm and a maximum at 195 nm, indicates significant amounts of β structure. The circular dichroism of the RNA indicates extensive regions of double helix. Aromatic Cotton effects, striking in their complexity, are detected in the near-ultraviolet circular dichroism of the isolated protein shells.

The theories of Crick and Watson (1956) and Caspar and Klug (1962) describe the general molecular organization of the simple isometric viruses. According to these theories, a minimum energy design for the 180 coat protein molecules of bacteriophage f2, a small single-stranded RNA virus of *ca.* 270 Å diameter, is an icosahedral protein shell of triangulation no. 3 (*cf.* review by Hohn and Hohn, 1970). The theories provide the framework for interpreting the characteristic appearance of isometric viruses in electron micrographs, and they provide the simple geometric arguments suggesting the importance of protein–protein and protein–nucleate interactions in virus structure; however, they provide neither insight into the nature of these interactions, nor information on the conformational constraints of one macromolecular component on the other. Although spectroscopic examination of the intact virus and its separated components, combined with hydrodynamic measurements of gross size and shape, provides a powerful means to study these aspects of virus structure, this approach has been seldom used. Consequently, we have measured the ultraviolet absorption, circular dichroism, and sedimentation velocity of bacteriophage f2, its separated protein shell or capsid component, and its RNA component. The capsids were obtained by short alkaline treatment (Kaper, 1960, 1964; Samuelson and Kaesberg 1970). Both RNA liberated during the alkaline treatment and RNA prepared by the phenol method (*cf.* Kaper, 1968) were examined.

Bacteriophage f2 was chosen for our structural investigation because it is relatively simple, and since its discovery by Loeb and Zinder (1961) it and its relatives (M12, MS2, fr, R17, and β) have been extensively studied, so that there is a wealth of information on the chemistry and molecular biology of the f2 group (*cf.* review by Hohn and Hohn, 1970). In addition, we were interested in obtaining spectral information which would aid the interpretation of our studies of the mechanism of neutralization of bacteriophage f2 by γG antibodies (Dudley *et al.*, 1970; Henkens *et al.*, 1971).

The molecular weight of the coat protein subunit of f2, 13,750, can be calculated with high accuracy from its known amino acid sequence (Weber and Konigsberg, 1967). In addition to the coat protein, bacteriophage of the f2 group contain one molecule of another protein, the maturation or A protein, of mol wt 38,000 (Steitz, 1968). Taking 180 coat protein molecules per virus particle and the weight fraction of RNA as 0.317 (*cf.* Kaper, 1968; Isenberg *et al.*, 1971), the mass of the virus particle, protein component, and RNA component can be calculated as 6.1×10^{-18}, 4.17×10^{-18}, and 1.94×10^{-18} g, respectively (the corresponding molecular weights are: 3.7×10^6, 2.5×10^6, and 1.16×10^6).

In as far as our work overlaps the spectroscopic characterization of μ2 (Isenberg *et al.*, 1971) and the hydrodynamic measurements of R17 (Samuelson and Kaesberg, 1970), most results are essentially the same. Although this is expected because of the similarity of f2, μ2, and R17, it allows a more sure combination and broader interpretation of experimental results.

In this study of f2 we report characterization of the size, shape, and folding of the capsid and RNA, and detection of complex aromatic Cotton effects, originating in the protein shell, which may be useful in studying subtle changes in molecular organization. Although it is not clear from our work what factors, other than steric, are responsible for maintaining the RNA strand inside the capsid, we observe that, at pH 11, it is readily released without extensive hydrolysis of the RNA or detectable changes in the secondary structure of the protein shell.

Materials and Methods

Materials. Bacteriophage f2 was purchased from Miles Laboratories Inc. It was obtained as a pH 7.6 buffered aqueous suspension (0.1 M NaCl–0.05 M Tris–0.001 M EDTA) containing 8–11 $\times 10^{14}$ particles/ml and stored at 4°. The phage titer, listed in an analysis supplied by Miles Laboratories with each batch, ranged from 1.0 to 2.0 $\times 10^{14}$ plaque-

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forming units per milliliter, depending on time elapsed since preparation. We commonly found significantly fewer plaque-forming units per milliliter, *ca.* 8×10^{12}, using the agar overlay technique (Adams, 1959) with either *Escherichia coli* K-37 or *E. coli* C-3000 (kindly supplied by Miles Laboratories) as the bacterial host. The sedimentation coefficient, $s^0_{20,w}$, of 79 S and the 280:260 nm absorbance ratio of 1.81 were similar to values previously reported (Loeb and Zinder, 1961; Hohn and Hohn, 1970); however, on occasion, about 15–30% ultraviolet-absorbing, λ_{max} 260 nm, nonsedimenting material was observed in the samples supplied by Miles. This material was removed by ultrafiltration. Electrophoresis of sodium dodecyl sulfate dissociated f2 on polyacrylamide gels, followed by staining with Coomassie Brilliant Blue, revealed two relatively sharp bands, a heavy one migrating with a mobility characteristic of a protein with molecular weight of the coat subunit and a trailing faint band migrating with a mobility characteristic of the A protein.

Cesium chloride was optical grade obtained from Schwarz-Mann. Guanidine hydrochloride was a product of Heico, Inc. (Delaware Water Gap, Pa.) and was used without further purification. Solutions were made with reagent grade NaCl and deionized water provided by Continental Water which had been filtered through a 0.45-μm Gelman membrane. The pH was adjusted with reagent grade HCl or NaOH.

Apparatus. Circular dichroism (CD) was measured with a Durrum-Jasco ORD/UV/CD-5 modified by Sproul Scientific Instruments (Boulder Creek, Calif.). The modifications, which include a change in the CD optics, an end-on photomultiplier, a redesign of amplifiers, and a change to 94-Hz Pockel cell modulation, combine to improve the signal-to-noise ratio by *ca.* 5, and result in an increase in the scale sensitivity by a factor of 6.6. Measurements were made near 25° in round fused silica cells of 1.0- or 10-mm path length. The total absorbance of the sample was always less than 2.0 in the wavelength region of interest. The slit width on the Durrum-Jasco instrument was programmed to give a spectral bandwidth of 2 nm. The instrument was standardized with a 1.00-mg/ml solution of *d*-10-camphorsulfonic acid using $[\psi]_{290} = 3.00 \times 10^3$ deg cm^3/(g dm). All CD results are reported in terms of ellipticity, ψ, which is directly related to the difference in absorbance of left and right circularly polarized light, $\psi = 33.0\ (A_L - A_R)$ (Lowry 1935). The specific ellipticity was calculated from

$$[\psi] = \psi/lc \qquad (1)$$

where ψ is the ellipticity in degrees, l is the path length in decimeters, and c is the concentration in grams per cubic centimeter. The mean residue ellipticity is

$$[\theta] = [\psi]M_0/100 \qquad (2)$$

where M_0 is the mean residue molecular weight. Values of 107 and 339 were used for the mean residue weights of protein and RNA components.

Absorbance measurements were made with a Cary 15 spectrophotometer which was periodically checked for absorbance linearity and excessive stray light. In addition, the wavelength calibration of the instrument was checked with benzene vapor, and its absorbance readings were checked with basic dichromate solution (West, 1960).

Sedimentation velocity measurements were made near 25° at 27,700 rpm on a Beckman-Spinco Model E analytical ultracentrifuge equipped with schlieren optics. Preparative ultracentrifugation employed the Beckman-Spinco Model L equipped with the SW-25.1 rotor with inserts for 6.6-ml centrifuge tubes.

Partial specific volumes were determined from densities measured with an Anton Paar DMA 02C digital density measuring device (Kratky *et al.*, 1969; Leopold, 1970) thermostated to better than ±0.01°.

Capsid Preparation. Capids were prepared by short alkaline treatment (pH 11.0) in 1.0 M NaCl solution according to the procedure of Samuelson and Kaesberg (1970). After 8 min, the treatment was terminated by adjusting to pH 7 with HCl. At this stage, ultracentrifugation of the reaction mixture revealed two components, the protein component with a sedimentation coefficient of approximately 41 S and the RNA component with a sedimentation of approximately 13 S. After digestion of the reaction mixture with RNase and gel filtration on Sephadex G-100 we added an additional purification step: the capsid fractions were pooled, made 35% w/v in CsCl, and centrifuged for 90–95 hr in the Spinco Model L at 22,500 rpm in 6.6-ml centrifuge tubes. Following centrifugation, the capsids were seen as a white band, 5–6 mm thick at 1.30 g/ml, bouyant density. They were used immediately or diluted with 1.0 M NaCl and stored at 4°.

The 280:260 nm absorbance ratio for our capsid preparations ranged from 1.66 to 1.55 indicating that they contained no more than 0.2–0.4% RNA contamination (Layne, 1957). In addition, we detected no phosphate (limits of detection about 0.25% RNA contamination) in our preparations by chemical analysis (Ames and Dubin, 1960). Banding in a CsCl gradient significantly reduces the RNA contamination of the capsids. Before this purification step, 280:260 nm absorbance ratios indicated 1–2% contamination. The importance of this increased purity for spectral studies of the capsids is illustrated in Figure 4 which shows the capsid aromatic Cotton effects. Here, 1.5% RNA contamination would represent approximately 32% of the total CD at 265 nm.

RNA Isolation. RNA was isolated from bacteriophage f2 by the phenol method (Schuster *et al.*, 1956; Gierer and Schramm, 1956) and used immediately. The RNA concentration was determined by phosphate analysis (Ames and Dubin, 1960) with intact f2 as standard.

Results

Ultraviolet Absorption Spectra of the Capsids. The ultraviolet absorption spectrum of the capsids, shown in Figure 1A, exhibits a peak at 278 nm and a minimum at 250 nm, characteristic of proteins containing tyrosine and tryptophan. A small scattering component was subtracted to obtain the true absorption spectrum (Winder and Gent, 1971). The specific absorptivity at 280 nm, using protein concentrations determined with Folin phenol reagent (Lowry *et al.*, 1951) with intact f2 as standard, is $E^{1\%}_{1cm} = 13.2$. Using a mol wt of 13,750, this corresponds to a molar absorptivity of ϵ_{280} 1.81×10^4 M^{-1} cm^{-1}. Since the f2 concentration was determined from its absorbance at 260 nm (Webster *et al.*, 1967) the absorptivity for the capsids is based on the absorptivity of intact f2 ($E^{1\%}_{1cm} = 76$ at 260 nm) and 0.68 weight fraction of protein. The absorptivity of the capsids at 280 nm is consistent with the tyrosine and tryptophan content of the coat protein, *viz.*, 4 and 2, respectively (Weber and Konigsberg, 1967). At 280 nm, the molar absorptivity of the coat protein in 6 M guanidine hydrochloride can be estimated from the tyrosine and tryptophan content as 5960 × number of tryptophans + 1280 × numbers of tyrosines = 1.70×10^4 (Edelhock, 1967). The mea-

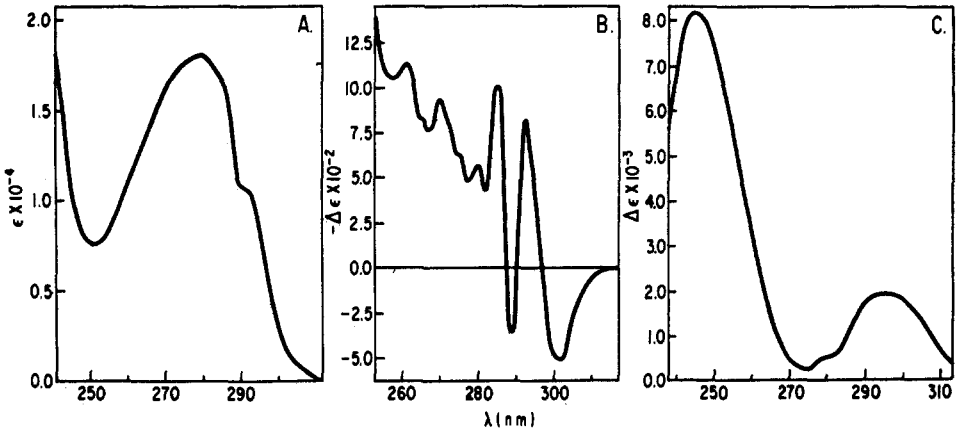

FIGURE 1: (A) Ultraviolet absorption spectrum of capsids near pH 6, 25° in 0.1 and 1.0 M NaCl. (B) Denaturation ultraviolet difference spectrum of capsids; the sample cell contained 6 M guanidine hydrochloride; both sample and reference cell contained the same concentration of protein, usually *ca.* 0.5 mg/ml in an unbuffered solution near pH 6, 25°. (C) Difference spectrum, pH 11 *vs.* pH 6, protein concentration *ca.* 0.8 mg/ml in 0.1 M NaCl.

sured change on guanidine hydrochloride denaturation is $\Delta A/A = -0.031 = \Delta\epsilon/\epsilon$ (Figure 1). Combining these values we calculate 1.76×10^4 M^{-1} cm^{-1} for the molar absorptivity of the coat protein, or $E^{1\%}_{1cm} = 12.8$.

Denaturation Ultraviolet Difference Spectrum. The ultraviolet difference spectrum resulting from the denaturation of the capsids by 6 M guanidine hydrochloride is shown in Figure 1B. The observed spectral changes are similar to those observed for the transfer of aromatic chromophores from an environment like the interior of a protein to an aqueous environment, although an anomalous peak is observed near 300 nm (*cf.* review by Donovan, 1969). The magnitude of the difference peak at 292 nm, assigned to a blue shift in the $\pi \rightarrow \pi^*$ absorption band of the indole chromophore of tryptophan residues, can be used to obtain a rough estimation of the number of tryptophan side chains transferred from an environment like the interior of a protein into the solvent as a result of the denaturation transition. The details of the calculation are outlined in a previous publication (Yazgan and Henkens, 1972). The observed change in molar absorptivity on denaturation is $\Delta\epsilon_{292} = -12.5 \times 10^2$. The change calculated for exposure of two buried tryptophan side chains per coat subunit is $\Delta\epsilon_{292} = -15.6 \times 10^2$. Comparison of the two values indicates that all tryptophan side chains (two per subunit) are buried in the virus structure and exposed during denaturation. The fine structure in the difference spectrum below 280 nm is indicative of the exposure of phenyl groups.

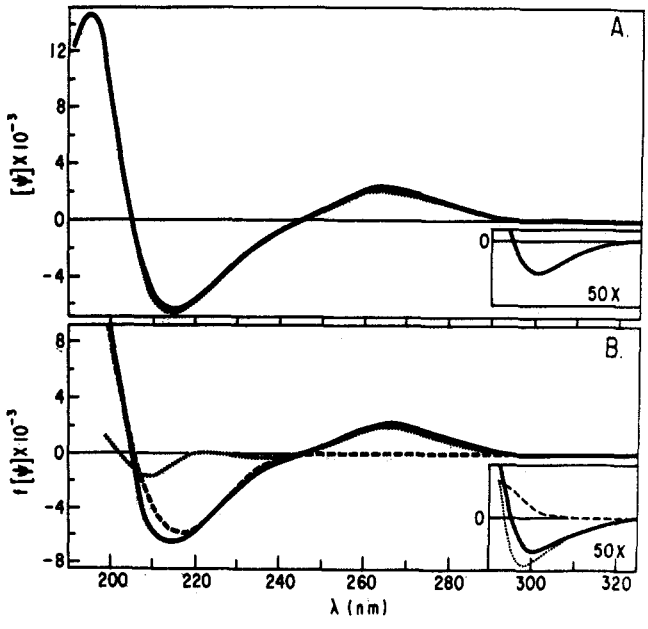

FIGURE 2: Circular dichroism near pH 6, 25°. (A) Bacteriophage f2 in 1.0 M NaCl, before (—) and after (· · ·) short alkaline treatment (see text). (B) Isolated protein shell (---) and RNA (· · ·); algebraic addition of partial specific ellipticities of isolated protein and RNA (—). Protein isolated after alkaline treatment; RNA isolated by phenol method (see text). Protein in 0.1 and 1.0 M NaCl; RNA in 1.0 M NaCl.

Ionization of Capsid Phenolic Side Chains. The near-ultraviolet absorption spectral changes observed on adjusting the capsid solution to pH 11.0 are characteristic of the spectral changes which occur when phenolic groups ionize (*cf.* review by Donovan, 1960). From the magnitude of the observed difference spectra (Figure 1C) we estimate that 150 tyrosine side chains (approximately 1 per coat subunit) are ionized at pH 11.0. Thus, of the four phenolic groups per subunit only one ionizes readily at pH 11.0. The others have abnormal pK_a values and so are evidently in some way inaccessible to the water molecules or hydroxyl ions.

Ultraviolet Circular Dichroism. The circular dichroism of bacteriophage f2, before and after a short alkaline treatment, shown in Figure 2A, contains major dichroic absorption bands at 266, 216, and 192 nm that are due to the protein and RNA components. Although the alkaline treatment results in complete release of the RNA (two bands are observed in the ultracentrifuge, one corresponding to protein and the other to RNA), there is almost no change in the ultraviolet (uv) circular dichroism.

Figure 2B shows the circular dichroism, in the region 190–320 nm, of the isolated protein shell and RNA components. The spectra are presented in terms of partial specific ellipticities, $f[\psi]$, where the specific ellipticity, $[\psi]$, is given by eq 1, and f is the weight fraction of the viral component. Algebraic addition of the circular dichroism curves for the protein and RNA components generates the curve shown, which is very similar to the circular dichroism of intact f2, indicating that, as far as can be judged by circular dichroism, isolation of the protein shell and RNA components does not alter their conformation.

Capsid far-Ultraviolet Circular Dichroism. The far-ultraviolet circular dichroism of the protein shell (Figure 3) con-

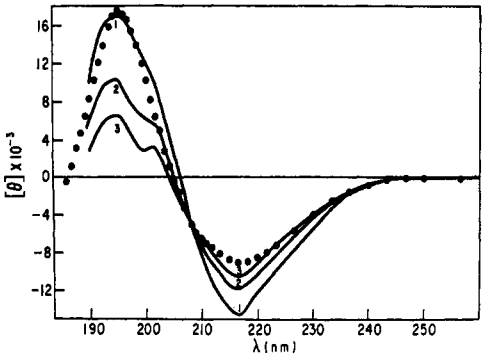

FIGURE 3: Far-ultraviolet circular dichroism of capsids (· · ·) in 0.1 and 1.0 M NaCl (region 320–200 nm), 0.1 M NaCl (region <200 nm), near pH 6, 25°, compared to curves calculated using β structure and random forms of poly(L-lysine) as standards (—): 1–80% β, 2–70% β, 3–65% β. Data for poly(L-lysine) standards are taken from Greenfield and Fasman (1969).

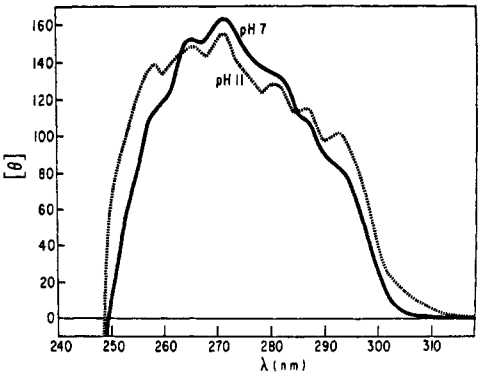

FIGURE 4: Near-ultraviolet circular dichroism of the capsids near pH 7 (—) and at pH 11 (---), 25°, in 0.1 and 1.0 M NaCl.

tains no bands characteristic of α-helical secondary structure. Although the circular dichroism of the β structure has not been as completely characterized as that of the α helix, the observed single minimum near 217 nm (as opposed to the double minimum at 208 and 222 nm for the α helix) and a maximum at 195 nm (as opposed to the maximum at 191 for the α helix) indicate the presence of this secondary structure (*cf.* Madison and Schellman, 1972; Timasheff, 1970; Greenfield and Fasman, 1969; Beychok, 1968). Using the β and random coil forms of poly(L-lysine) as standards for 100% β structure and random coil, we estimate roughly 60% β structure and 40% random coil for the protein of bacteriophage f2. In fitting the experimental curve (Figure 3) with poly(L-lysine) standards, we ran into the usual difficulty at lower wavelength (Greenfield and Fasman, 1969), so these wavelengths we excluded. A much better fit of the lower wavelength positive band is obtained using β structure and "nonperiodic" spectra derived from protein X-ray diffraction data and experimental circular dichroism (Saxena and Wetlaufer, 1971). This basis for interpreting the circular dichroism also gives 60% β, but predicts a small peak at 235 nm which is not observed, and a band at 220 nm rather than the band at 217 nm actually observed. The best fit of the experimental circular dichroism of f2 capsids is obtained using poly(L-lysine) standard curves for β, and curves characterized by a single small negative band centered near 200 nm (Tiffany and Krim, 1969) for the nonperiodic. On this basis we calculate 50% β and the rest random or nonperiodic. However, it should be recognized that even moderately accurate determination of per cent β structure in proteins from circular dichroism is very uncertain at present; the technique is much more powerful in its ability to monitor changes in structure. No changes in the far-ultraviolet circular dichroism of the capsid are detected when the pH is adjusted from pH 7 to 11, indicating that the alkaline treatment does not importantly affect the protein secondary structure.

Capsid Aromatic Cotton Effects. The circular dichroism of the capsid, in the near-ultraviolet region, 250–310 nm, shown in Figure 4, contains a number of dichroic absorption bands that are most likely due to tryptophan and tyrosine residues, although phenyalanine may also contribute (*cf.* Timasheff, 1970). Since these aromatic side chains all contain a plane of symmetry, any circular dichroism contribution from these chromophores must result from disymmetric interaction with other groups of atoms.

Small amounts of contaminating RNA could contribute to the circular dichroism in this region of the spectrum; indeed as mentioned previously, 1.5% RNA contamination would contribute about one-third of the total dichroic absorption in this region. However, analysis shows less than 0.4% RNA. In addition, denaturation of the capsid in 6 M guanidine hydrochloride abolishes the dichroic absorption in this region, whereas 6 M guanidine hydrochloride causes only a red shift and moderate decrease in the circular dichroism of RNA. Thus, contaminating RNA evidently does not make a detectable contribution to the capsid aromatic Cotton effects.

Small changes in the aromatic Cotton effects are observed when the pH is raised from pH 7 to 11 (Figure 4); there is an increase in dichroic absorption around 292 nm, where a band due to ionized tyrosine has been identified, and a decrease around 275 nm, where there is a band due to un-ionized tyrosine. This suggests that the observed changes are due, in part at least, to the ionization of tyrosine side chains (*cf.* Timasheff, 1970).

RNA Circular Dichroism. RNA isolated from bacteriophage f2 by the phenol method exhibits intense, nonconservative circular dichroism. The spectrum (Figure 5), which has a positive band centered at 265 nm, a very small band at longer wavelength (insert Figure 5), and additional positive and negative bands at shorter wavelengths, is chracteristic of RNA molecules with double helical parts (*cf.* review by Yang, 1968; Gratzer and Richards, 1971). Analysis of the circular dichroism in terms of two conformations, double stranded with the spectral characteristics of RNA double helix, and single stranded with base stacking, according to the method of Gratzer and Richards (1971) shows that there is extensive

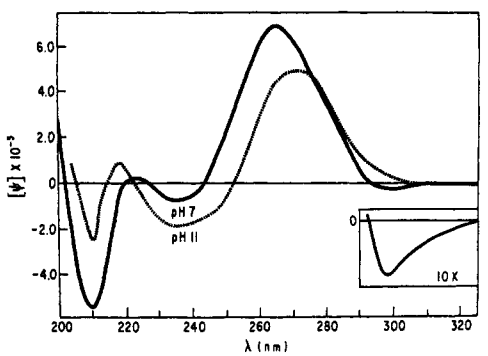

FIGURE 5: Circular dichroism of RNA from bacteriophage f2 isolated by the phenol method near pH 7 (—) and pH 11 (---), 25°, in 1.0 M NaCl.

TABLE I: Sedimentation and Frictional Coefficients of Bacteriophage f2 and Isolated Protein Shell.[a]

	Mol Wt	$\bar{\nu}_2$	$s^0_{20,w} \times 10^{13}$	$F \times 10^7$	δ_1
Intact f2	3,670,000	0.667	79.2 ± 0.6	2.6	0.86
Protein	2,510,000	0.706 ± 0.007	41.4 ± 0.3	3.0	3.14

[a] Measurements of s and $\bar{\nu}_2$ were made in 0.10 M NaCl, pH 6; the standard error is included for cases where the set of measurements contains five or more values.

base pairing in the f2 RNA molecule. Short treatment at pH 11 results in essentially reversible changes in the circular dichroism (Figure 5), very like the changes calculated for a decrease in the per cent base pairing.

Partial Specific Volume. The partial specific volumn, $\bar{\nu}_2$, of intact f2 and of the capsids was determined from density measurements made with the Anton Paar DMA 02C digital density measuring device (Kratky *et al.*, 1969; Leopold, 1970) using the formula

$$\bar{\nu}_2 = \frac{1}{c}\left(\frac{\rho_0 - \rho}{\rho_0}\right) + \frac{1}{\rho_0} \qquad (3)$$

where c is the concentration of solute, ρ is the density of the solution, and ρ_0 is the density of the solvent. The concentration, which ranged from 2.5×10^{-3} to 5.0×10^{-4} g/cm^3, was determined from absorbance measurements near 23°. The density measuring device was thermostated to better than ±0.01° at 21.3°.

The partial specific volumes of intact f2 and capsids, in 0.1 M NaCl, pH near 6, are given in Table I. We did not detect any trend of $\bar{\nu}$ with concentration. The value for intact f2, $\bar{\nu}_{f2}$ = 0.667 cm^3/g, is similar to values previously reported for f2 group phage (Samuelson and Kaesberg, 1970). The partial specific volume of the capsids, $\bar{\nu}_{capsid}$ = 0.706 cm^3/g, is significantly different from the value of 0.738 cm^3/g calculated for f2 coat protein using the procedure of Cohn and Edsall (1943).

Sedimentation. The capsids and intact f2 sediment, in the analytical ultracentrifuge, as a single symmetrical boundry. Sedimentation coefficients, measured in 0.10 M NaCl at five concentrations ranging from 0.3 to 1.8 mg/ml, were reduced to standard conditions, and extrapolated to zero concentration, assuming a linear variation of $s_{20,w}$ with concentration (the slope of $s_{20,w}$ *vs.* concentration was small for both f2 and capsid). The resulting $s^0_{20,w}$ values are given in Table I. Frictional coefficients, F, were calculated from

$$F = \frac{M(1 - \bar{\nu}_2\rho_0)}{Ns^0} \qquad (4)$$

taking the molecular weight, M, of intact f2 and capsids as 3.67×10^6 and 2.51×10^6, and are given in Table I.

The RNA liberated by the alkaline treatment sediments as a broad symmetrical front with an $s_{20,w}$ value of 12 S (1.0 mg/ml of RNA, 1.0 M NaCl, pH 6), suggesting it is not extensively hydrolyzed; in addition, heating to 70° for 15 min, followed by rapid cooling, causes only a small decrease (less than 1 Svedberg unit) in the sedimentation coefficients (the heat treatment precipitates the protein; however, the precipitate sediments rapidly in the ultracentrifuge, and evidently causes no interference).

Gel Electrophoresis. Polyacrylamide gel electrophoresis of sodium dodecyl sulfate dissociated f2, followed by staining with Coomassie Brilliant Blue, clearly reveals a sharp band migrating with the mobility characteristics of the A protein. No such band is observed on electrophoresis of our capsid preparation. Evidently the A protein is lost during preparation of the capsids.

Discussion

Short alkaline treatment of bacteriophage f2 results in liberation of the RNA, leaving the protein shell of the virus essentially intact as judged by our optical and hydrodynamic measurements. Electron micrographs show hollow spherical particles of approximately 135-Å radius (Hohn and Hohn, 1970). Assuming a mol wt of approximately 2.5×10^6, the particles behave hydrodynamically as solvent-filled solid shells. The shell is evidently not *free draining*. From a hydrodynamic point of view the solvent is trapped (*cf.* Tanford, 1961), although the surface lattice of protein subunits undoubtedly has holes through which solvent can enter and leave. The frictional coefficient of the capsid is somewhat more than that of the intact virus particle (Table I) indicating some difference in particle shape or dimensions.

Assuming that the shapes of f2 and of capsid can be roughly approximated by a rigid sphere, we calculate 138 and 156 Å for the radius of the hydrated sphere corresponding to f2 and to capsid, respectively, using the Stokes' equation for the frictional coefficient (*cf.* Hohn and Hohn, 1970; Zipper *et al.*, 1971). The corresponding volume, ν_h, can be expressed in terms of the thermodynamic partial specific volume, $\bar{\nu}_2$, and a solvation factor δ_1 which represents the grams of solvent associated with 1 g of unsolvated particle (Tanford, 1961). ν_1^0 is the specific volume of pure solvent. The

$$\nu_h = (M/N)(\bar{\nu}_2 + \delta_1\nu_1^0) \qquad (5)$$

value of δ_1 for f2 and capsid is 0.86 and 3.14 respectively. We assume that a large proportion of the solvent associated with the capsid is simply trapped within the shell.

The ultraviolet circular dichroism of bacteriophage f2 (Figure 2) displays three major Cotton effects associated with the protein and nucleic acid components. The longest wavelength Cotton effect is due almost entirely to RNA, while the shorter wavelength ones are dominated by the protein. In addition, there is a very small band near 300 nm (insert of Figure 2) which is due primarily to the RNA, but which also contains a significant contribution from the long-wavelength tail of the protein aromatic Cotton effects.

Although the aromatic Cotton effects in the near-ultraviolet circular dichroism of the capsid (Figure 4) are small relative to the Cotton effects arising from optically active peptide transitions, they are striking in their complexity, and large compared to the dichroic absorption of free aromatic amino acids on their simple derivatives (Legrand and Viennet, 1965; Beychok, 1966). Since the Cotton effects must result from disymmetric interaction of the aromatic side chains with neighboring groups of atoms, it is expected that they will be sensitive to tertiary and even quarternary structure, so that they may be useful in monitoring small changes in the molecular organization of the capsid. Unfortunately, the near-uv region is dominated by RNA circular dichrosim and so

the aromatic Cotton effects cannot readily be used to study protein–nucleate interactions.

The slight increase in frictional coefficient on liberation of RNA from f2, detected by hydrodynamic measurements, is evidently not accompanied by changes in secondary structure of the protein subunits, nor does any hydrolysis of the RNA strand affect its base pairing and stacking as judged by circular dichroism. Isenberg *et al.* (1971) report a high degree of base pairing in $\mu 2$ RNA, estimated at $63 \pm 5\%$ from an analysis of infrared spectra. Our circular dichroism spectra are certainly consistent with this value, although we have not attempted detailed analysis.

While the present study does not provide any detailed information on protein–nucleate interactions in RNA viruses, the results show that any interactions between RNA and protein in bacteriophage f2 do not significantly affect the circular dichroism of either component. This is in marked contrast to the DNA phages where the DNA Cotton effects differ greatly from those of free DNA (Maestre and Tinoco, 1967).

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