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Comparison of Two Serologically Distinct Ribonucleic Acid Bacteriophages

I. Properties of the Viral Particles

L. R. OVERBY,[1] G. H. BARLOW[1], R. H. DOI,[2] MONIQUE JACOB,[3] AND S. SPIEGELMAN

Department of Microbiology, University of Illinois, Urbana, Illinois, and Biochemistry Research Department, Abbott Laboratories, North Chicago, Illinois

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ABSTRACT

OVERBY, L. R. (University of Illinois, Urbana), G. H. BARLOW, R. H. DOI, MONIQUE JACOB, AND S. SPIEGELMAN. Comparison of two serologically distinct ribonucleic acid bacteriophages. I. Properties of the viral particle. J. Bacteriol. **91**:442–448. 1966.—Two ribonucleic acid (RNA) coliphages, MS-2 and Qβ, have been characterized physically and serologically. MS-2 has an $S_{20,w}$ value of 79, a molecular weight of 3.6×10^6, a density of 1.422, and *p*H 3.9 as its isoelectric point. Qβ has an $S_{20,w}$ of 84, a molecular weight of 4.2×10^6, a density of 1.439, and an isoelectric point at *p*H 5.3. One host (*Escherichia coli* A-19) permits a distinction between the two on the basis of a marked difference in plaque size. They are distinct immunochemically, no serological cross-reaction being detectable.

Since the original discovery of the f2-coliphage by Loeb and Zinder (13), a number of ribonucleic acid (RNA) bacteriophages specific for *Escherichia coli* have been independently isolated and studied. These include Alvin J. Clark's MS-2 (21), R-17 (6, 7, 18), M-12 (10), fr (15), and β (17). Recently, Bishop and Bradley (3) isolated six ribonucleic acid (RNA) coliphages which fell into two groups serologically. However, their relation to the ones reported earlier was not determined. Nonoyama, Yuki, and Ikeda (17) established some degree of serological relatedness to MS-2 of 13 different isolates. In confirmation, Scott (20) used antisera against f2 and MS-2 to show cross-reactions among f2, MS-2, fr, M-12, R-17, and β, which includes all those employed to study RNA replication. Initial studies in this laboratory (4, 5, 8) were carried out with MS-2. For a variety of obvious reasons, it was highly desirable to perform parallel experiments with two unrelated RNA viruses. One could then hope to detect and employ differences in RNA, coat protein, and the enzymes induced in the course of the infection. The discovery by Watanabe (22) of a new RNA phage, Qβ, apparently unrelated to any of those specifically noted above, made such a comparative study possible. In particular, it permitted the demonstration (9) that the RNA replicases induced by MS-2 and Qβ were distinct, each exhibiting a unique preference for its homologous RNA as a template. When properly purified, and under optimal ionic conditions, both enzymes were virtually inactive with heterologous RNA, including ribosomal and soluble RNA of the host. Finally, neither replicase could function with the other's RNA, each recognizing the RNA genome of its origin and requiring it as a template for synthetic activity.

In the course of these studies, a considerable amount of information on the properties of these viruses and their components has accumulated. It is the primary purpose of the present paper to summarize the available information on the physical characteristics of MS-2 as compared with Qβ. A subsequent communication will detail the properties of the RNA and coat protein of each.

MATERIALS AND METHODS

Viruses, hosts, and medium. The bacterial viruses employed were MS-2 (originally obtained from A. J. Clark) and Qβ (kindly provided by I. Watanabe). The following Hfr strains of *E. coli* were used: K-10 (Garen), A-19 (a mutant lacking ribonuclease I, described by Gesteland, Federation Proc. **24**:293, 1965),

[1] Present address: Abbott Laboratories, North Chicago, Ill.

[2] Present address: Department of Biochemistry and Biophysics, University of California, Davis.

[3] Present address: Institut de Chimie Biologique, Faculté de Médicine, Strasbourg, France.

and Q-13, a derivative of A-19 which also lacks RNA phosphorylase, isolated by Diane Vargo in the laboratory of W. Gilbert at Harvard University.

General growth medium (L medium) was, in grams per liter: glucose, 1.0; tryptone (Difco), 10.0; yeast extract, 5.0; NaCl, 10.0; plus 1.0 ml of 2 M $CaCl_2$. A synthetic medium (SC) was used for preparing P^{32}- and uridine-H^3-labeled phage. It contained 0.055 M NH_4Cl, 0.170 M NaCl, 0.054 M KCl, 0.006 M $MgSO_4$, 0.0005 M Na_2HPO_4, 0.0006 M $FeCl_3$, 0.011 M glucose, and 0.0002 M $CaCl_2$ in 0.01 M tris(hydroxymethyl)aminomethane (Tris; *p*H 7.2). The general buffer was TM: 0.01 M Tris (*p*H 7.2), 10^{-4} M $MgCl_2$.

Growth and purification of phage. Both phages were produced by a generally applicable technique. A suspension of *E. coli* K-10 growing in early log phase (2.5×10^8 cells per milliliter) at 37 C in L medium was inoculated at a multiplicity of 0.1, and aeration continued for 6 to 8 hr when lysis became evident. Unlysed cells were removed by centrifugation, ruptured with lysozyme and chloroform, and the released phage was combined with the original lysate. The combined supernatant fluid was made 2.0 M with solid $(NH_4)_2SO_4$ and kept at 4 C for 4 hr. The precipitated phage was collected by centrifugation at 15,000 $\times$ *g* for 30 min, suspended in a minimal volume of TM buffer, and dialyzed against two changes of the same buffer. The crude phage suspension was purified by centrifugation in a 5 to 20% sucrose density gradient buffered with 0.01 M Tris, as described below. Phage particles were readily separated from 30*S* and 50*S* ribosomes and low molecular weight contaminants. The low concentration of Mg^{++} eliminated 70*S* ribosomes and their aggregates. The phage peak, located by absorbancy and plaque-forming ability, was pooled and concentrated by a second ammonium sulfate precipitation.

P^{32}-labeled phage was prepared as follows: cells were grown in SC medium containing 5 to 10 mc of P^{32}-labeled orthophosphate per 100 ml of culture for two generations prior to infecting with phage at a multiplicity of 0.1. Isolation and purification were as described above. The final preparation was dialyzed against three changes of 0.01 M phosphate buffer (*p*H 6.7). Specific activity of the phage was of the order 10^{-6} count/min per particle.

H^3-labeled phage was made in SC medium. Uridine-H^3 was added at a level of 5 μg and 50 μc per ml 15 min prior to infecting at a multiplicity of 5. Isolation and purification as above yielded phage of about 10^{-7} count/min per particle.

Assay. The standard two-layer agar technique for plaque-forming units (PFU) was used (1). The bottom layer was L medium plus 1.5% agar (Difco), and the top layer was L medium plus 1% agar (Difco). Indicator bacteria were an overnight culture grown in L medium. Antiserum was prepared in rabbits and assayed by the procedure described by Adams (1).

Density-gradient centrifugation. Concentrated phage suspensions were layered on 5 to 20% sucrose gradients in TM buffer, and centrifuged for 4 hr at 4 C and 25,000 $\times$ *g* in the SW 25.1 rotor of the Spinco model L ultracentrifuge. Samples were collected from the bottom of the tube and analyzed for absorbance at 260 mμ, radioactivity, and PFU.

Electrophoresis. Analyses were carried out in a Spinco model H electrophoresis-diffusion apparatus by use of the 2-ml micro cell. Samples (5 mg/ml) were dialyzed overnight in the appropriate buffer systems: 0.01 M Tris (*p*H 7.4), 0.01 M acetate (*p*H 5.0), and 0.01 M glycine-HCl (*p*H 2.1).

Molecular weight determinations. Phage particles were suspended in 0.01 M Tris (*p*H 7.2) plus 0.1 M NaCl at a concentration of 0.5 to 1.0% for all procedures relating to molecular weight estimation by the following methods.

Sedimentation velocity. Sedimentation data were obtained with a Spinco model E ultracentrifuge. The runs were made at 15,200 $\times$ *g* and 20 C. Calculation of sedimentation coefficients were made by the method of Schachman (19). The partial specific volumes ($\bar{v}$) were determined by the zero sedimentation method with D_2O-H_2O mixtures of different compositions and densities as described by Katz and Schachman (11). The *S* values thus obtained were corrected for deuterium-hydrogen exchange by the method of Martin, Winkler, and Cook (14). Diffusion coefficients (*D*) were determined in a Spinco model H electrophoresis-diffusion apparatus at 1 C. Calculations were made from Rayleigh fringe patterns and corrected to 20 C by standard methods (19).

Light scattering. Measurements were made on a Brice-Speiser-Phoenix light-scattering photometer with light of 5,436 A (2). Dust and large particulate matter were first removed by filtration through type HA Millipore filters. It was observed that these filters had a high capacity for retaining phage, especially Qβ. The filters were first "saturated" with phage and

TABLE 1. *Phage yield and efficiency of plating with three strains of Escherichia coli**

Bacteria used for virus growth	Bacteria used for virus assay					
	K-10		A-19		Q-13	
	MS-2	Qβ	MS-2	Qβ	MS-2	Qβ
K-10	1.0×10^{12}	6.3×10^{11}	8.5×10^{11}	1.2×10^{12}	1.2×10^{12}	2.1×10^{12}
A-19	1.4×10^{12}	6.6×10^{11}	1.1×10^{12}	1.3×10^{12}	1.6×10^{12}	1.9×10^{12}
Q-13	6.3×10^{11}	1.1×10^{11}	5.1×10^{11}	2.3×10^{11}	6.7×10^{11}	3.7×10^{11}

* Numbers represent plaque-forming units per milliliter.

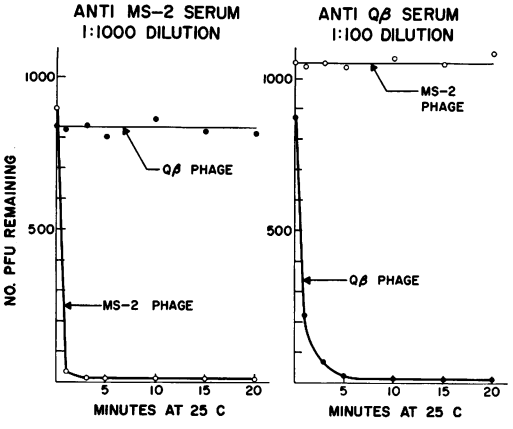

FIG. 1. *Comparison of interaction of MS-2 and Qβ phages with heterologous antisera. Phage (0.1 ml; 10^6 PFU) was treated with 0.9 ml of diluted antiserum in L medium at 25 C. At intervals, 0.1 ml of phage-serum mixture was added to 9.9 ml of L medium, and 0.1 ml of this dilution (10^3 PFU) was plated in duplicate.*

then used for cleaning the samples for light scattering. The binding of phage by cellulose-nitrate filters has since been reported by Lodish and Zinder (12). The refractive increment (*dn/dc*) was determined in a Phoenix differential refractometer. All concentrations were estimated by absorbancy at 260 mμ. Specific absorbancy was determined by weighing dried samples of solutions with known optical density (OD).

RESULTS

Growth in different hosts. The yield and plating efficiency of the two viruses on each of three Hfr strains of *E. coli* were examined and the results are summarized in Table 1. In general, the burst size of MS-2 is twice that of Qβ. The ribonucleaseless mutant (A-19) gives the highest titer of lysates. However, Q-13, which also lacks RNA phosphorylase, yields the highest efficiency of plating (EOP). The difference is most strikingly seen with the virus Qβ where the EOP on K-10, A-19, and Q-13 is as 1:2:3, respectively. The A-19 strain can be used for a quick diagnostic distinction between Qβ and MS-2, since the latter yields uniformly minute plaques when plated on A-19, whereas Qβ produces the usual plaques seen on any other host tested.

Serological relatedness. As noted by Watanabe (*personal communication*), there was little detectable serological cross-reaction between the two viruses (Fig. 1). When used as an antigen, MS-2 readily produces in rabbits antisera possessing velocity constants (*K*) of 1,000 to 2,000 at 25 C. On the other hand, Qβ is a much less potent antigen, yielding antisera with *K* values of about 100. The two phages appear to be unrelated serologically.

Size and shape of purified preparations. The electron micrographs of purified preparations shown in Fig. 2 indicate the homogeneity of the

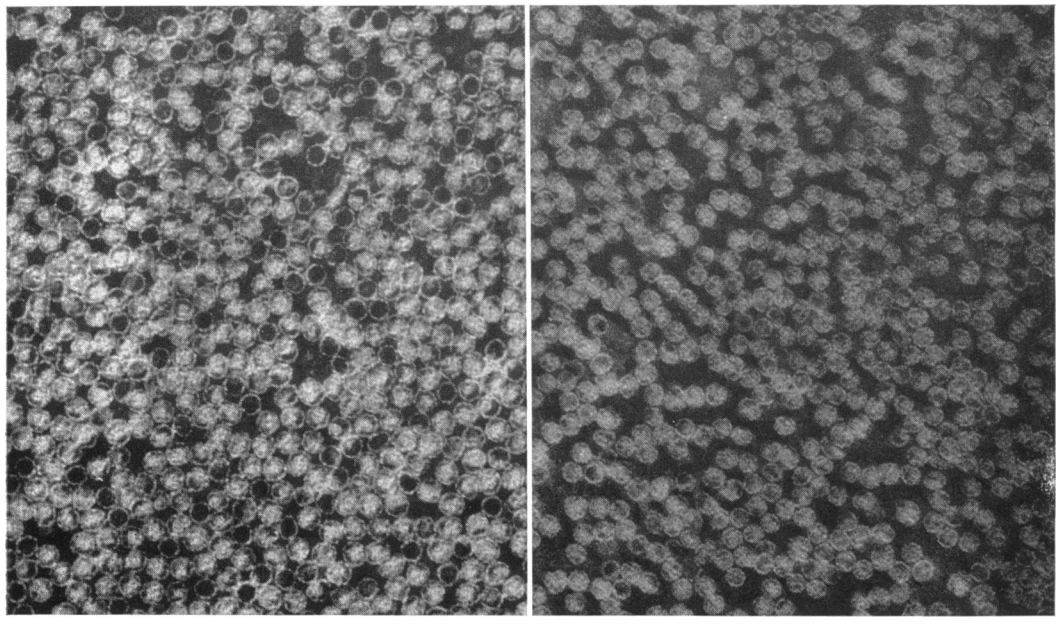

FIG. 2. *Electron micrographs of Qβ (left) and MS-2 (right) phages negatively stained with phosphotungstate; final magnification, × 100,000.*

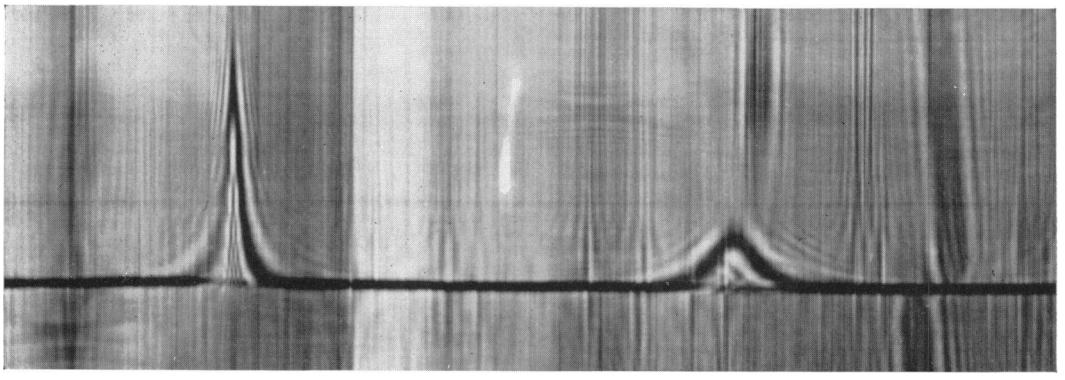

FIG. 3. *Separation of a mixture of MS-2 (left pattern) and Qβ (right pattern) coliphages in model H electrophoresis apparatus. Concentration of each phage was 0.25% in 0.01 M Tris (pH 7.5) and 0.1 M NaCl. Picture of the ascending limb was taken 59.3 min after electrophoresis at 6 v/cm; starting boundary at right margin; migration toward anode (left margin).*

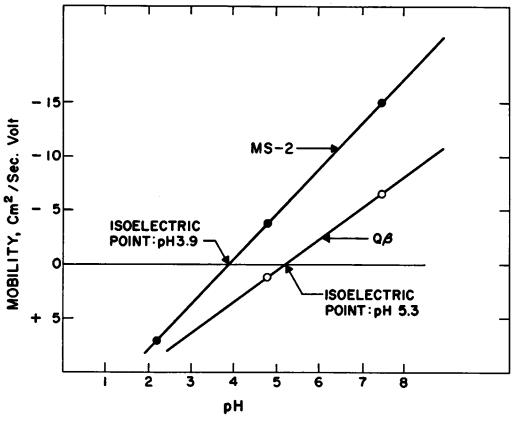

FIG. 4. *Plot of electrophoretic mobility of MS-2 and Qβ phages vs. pH of buffer.*

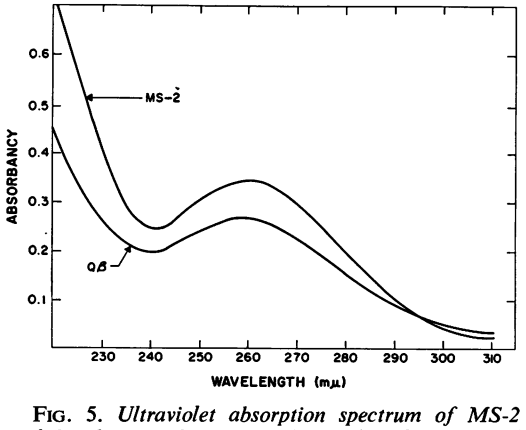

FIG. 5. *Ultraviolet absorption spectrum of MS-2 and Qβ phages in 0.01 M Tris (pH 7.2) and 0.1 M NaCl at 25 C.*

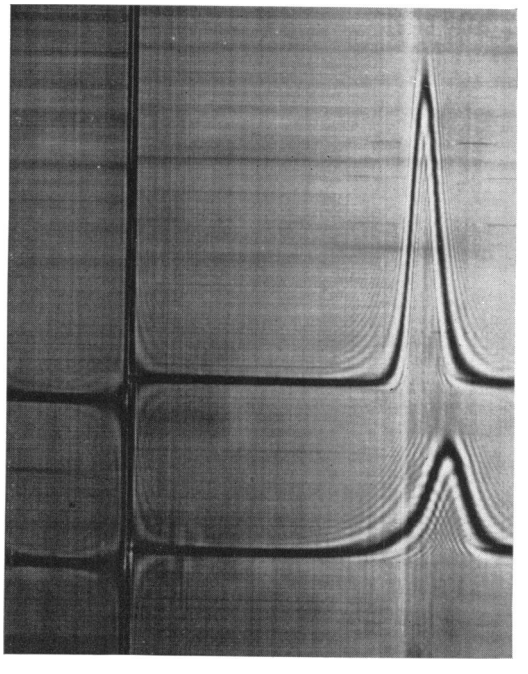

FIG. 6. *Comparative sedimentation patterns of MS-2 (upper) and Qβ (lower) phages in model E ultracentrifuge, with use of two 30-mm standard cells, one with wedge window. Concentrations of phage were 0.5% in 0.01 M Tris (pH 7.3) and 0.1 M NaCl. Picture taken 86 min after reaching a speed of 15,220 × g at 20 C.*

preparations used in these studies and also the virtual identity of the two viruses in size, shape, and appearance. Both are polyhedrons with a diameter of about 25 mμ.

Isoelectric points. The surface charge of the

TABLE 2. *Observed sedimentation coefficients of phages as a function of D_2O-H_2O mixtures of different densities at 25 C*

Phage	D_2O	Density	Viscosity	$S_{obs} \times 10^{13}$	K^*
	%	g/ml	η	sec	
MS-2	94.0	1.109	1.160	53.3	1.014
	88.6	1.103	1.148	56.0	1.013
	69.1	1.083	1.106	59.5	1.010
	44.3	1.056	1.054	70.5	1.006
	0	1.010	0.960	82.5	1.000
Qβ	94.0	1.109	1.160	69.8	1.014
	89.1	1.104	1.149	70.9	1.013
	69.1	1.083	1.106	75.1	1.010
	24.9	1.036	1.012	83.2	1.003
	0	1.010	0.960	87.9	1.000

* Constant to correct for deuterium exchange with phage hydrogen (14).

two particles is quite different, and they are easily separated by electrophoresis. Figure 3 shows an electrophoretogram of a mixture of the two purified preparations at *p*H 7.5. The higher negative charge of MS-2 and the greater diffusion of Qβ are clearly evident. Electrophoresis at different *p*H values gave the results shown in Fig. 4. The empty particles seen, particularly with Qβ, are not detected as second components in analytical ultracentrifugal runs (Fig. 6), which suggests that they occur during processing for electron microscopy. The isoelectric points were calculated to be 3.90 for MS-2 and 5.3 for Qβ.

Ultraviolet spectrum. The ultraviolet-absorption spectrum showed no striking differences (Fig. 5). MS-2 possessed a higher absorbancy in the 220 to 230 mμ range, probably reflecting a different amino and composition in the coat protein. The specific absorbancies determined by weighing the dry solids in samples of known OD at 260 mμ were 7.81 per mg per ml for MS-2 and 8.07/mg per ml for Qβ. The composition of both particles was approximately 25 to 30% RNA and 70 to 75% protein.

Molecular weights. Molecular weights of the particles were determined by light scattering and by sedimentation velocity in the ultracentrifuge. The envelopes of both phages were determined at 546 mμ at five different concentrations. The refractive increments, determined in the differential refractometer, were 0.199 ml/g for MS-2 and 0.198 ml/g for Qβ. Within experimental error, the intercepts were independent of concentration. From the intercepts, the particle molecular weights of MS-2 and Qβ were 3.6×10^6 and 4.2×10^6, respectively. Measurements were also carried out at 436 mμ with MS-2, and an identical molecular weight of 3.6×10^6 was obtained.

Figure 6 shows a simultaneous ultracentrifuge run with both phages. The samples gave symmetrical patterns with $S_{20,w}$ values of 78.5 for MS-2 and 84.3 for Qβ.

Diffusion coefficients were calculated from Rayleigh fringe pictures in a diffusion apparatus at 1 C, and corrected to 20 C as $D_{20,w}$. The values were 1.64×10^{-7} cm² per second for MS-2 and 1.55×10^{-7} cm² per second for Qβ.

The partial specific volume, $\bar{v}$, is needed for calculation of molecular weight from sedimentation measurements. Conventional methods for determining $\bar{v}$ usually employ pycnometers or density determination in gradients of cesium salts. Large concentrations of phage are required for accurate weighings in a pycnometer. The possibility of interaction of cesium salts with the virus may give inaccurate densities when this method is used. These limitations are obviated by use of the zero sedimentation method of Katz and Schachman (11) with results as shown in Table 2. In this procedure, sedimentation coefficients are measured in D_2O-H_2O mixtures of different composition and density. A suitable function of S is extrapolated to give the density at which the particle possesses zero sedimentation rate. This method is restricted to a two-component system, since D_2O-H_2O acts as a one-component solvent. Extrapolation of the observed S values by a line of least squares gave densities of 1.422 and 1.439. The partial specific volumes, $\bar{v}$, are the inverses, and correspond to 0.703 and 0.695 for MS-2 and Qβ, respectively.

Molecular weights were calculated by the Svedberg equation $M = \frac{RTS}{D(1-\bar{v}\rho)}$ where D is diffusion coefficient; S, sedimentation coefficient; $\bar{v}$, partial specific volume; T, absolute temperature; R, the gas constant; and ρ, the solvent density. The usual assumption was made that sedimentation and diffusion possess similar concentration dependence. The molecular weights were 3.87×10^6 for MS-2 and 4.29×10^6 for Qβ, in good agreement with the light-scattering results.

DISCUSSION

Comparisons of the available data on the physical and hydrodynamic properties of RNA bacteriophage particles are summarized in Table 3. It will be noted that the values for S, density, and diameter found by Watanabe (*personal communication*) for Qβ are in excellent agreement with those found in the present study. Similarly, there are no discrepancies with the

TABLE 3. *Summary of reported physical and hydrodynamic properties of seven Escherichia coli RNA bacteriophages*

Phage	Isoelectric point (pH)	Particle diam	Specific absorbancy*	$S_{20,w}$	Refractive increment	Molecular wt	Density	$D_{20,w}$	Partial spec. volume ($\bar{v}$)	References
		mμ			ml/g	g	g/ml	cm^2/sec	ml/g	
MS-2	3.9	25	7.81	79	0.199	3.7×10^6	1.422	1.64×10^{-7}	0.703	Present publication
MS-2	—	26	8.03	81	0.191	3.6×10^6	1.46	—	0.690	(21)
Qβ	5.3	25	8.02	84	0.198	4.2×10^6	1.439	1.55×10^{-7}	0.695	Present publication
Qβ	—	24	—	85	—	—	1.43	—	—	Watanabe (*personal communication*)
f2	—	20	—	—	—	—	—	—	—	(13)
R-17	—	—	7.66	80	0.182	3.6×10^6	—	—	—	(7)
R-17	—	—	8.20	77	0.177	4.2×10^6	—	1.328×10^{-7}	0.670	(6)
M-12	—	27	—	—	—	—	—	—	—	(10)
β	—	—	—	90	—	—	—	—	—	(17)
fr	9	21	7.60	79	—	4.1×10^6	1.46	1.45×10^{-7}	0.690	(16)

* Optical density at 260 mμ.

previously reported data of Strauss and Sinsheimer (21) on MS-2.

With the exception of the isoelectric point, the values found for the different coliphage strains fall within the limits of experimental error. There appears to be a wide variation in surface charge, as indicated by the three reported isoelectric points of 3.9 (MS-2), 5.3 (Qβ), and 9 (fr). Since all possess the same ratio of RNA to protein, this difference in surface charge may be ultimately explained in the nature of the coat protein and, possibly, its physical relation to the RNA.

Strauss and Sinsheimer (21) commented on the observation that experimentally determined molecular weights of small spherical RNA viruses are about one-half the value estimated from density measurements in CsCl and particle volumes calculated from electron microscope pictures. A suggested explanation was that the particles are permeable to, or interact with, CsCl, the medium in which densities are usually determined. To avoid this difficulty, the densities of the virus particles were determined in D_2O-H_2O mixtures, a two-component system. The values found by this method, 1.42 to 1.43, are only slightly lower than the CsCl value of 1.46, indicating very little interaction of viral particle with CsCl. Marvin and Hoffmann-Berling (16) found for f2 about 1 g of water of hydration per g of phage from viscosity and diffusion measurements. Should this water fill internal voids, it is likely that results from sedimentation would disagree correspondingly with estimates derived from electron microscopic visualization.

Though physically quite similar, there is no doubt that MS-2 and Qβ are different chemically. This is suggested by the ease with which they can be distinguished immunochemically. In addition, as noted, it has been demonstrated (9) that the RNA replicases they induce are readily differentiated on the basis of the sequences each enzyme recognizes and accepts as a template. Finally, the next paper in this series will offer evidence for difference in the chemical composition of both RNA and coat protein of these two viruses.

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