binds the 5′-terminal NTP. This conclusion is based on the similarity of the apparent K_m of initiation and the dissociation constant, on the specificity of the first site to bind purine nucleotides, and on the effect of rifamycin. From the fluorescent studies (Wu and Goldthwait, 1969) it can be concluded that this site is not on the σ protein (Burgess *et al.*, 1969).

The nature of the second site observed by equilibrium dialysis is less certain. Although its dissociation constant is comparable with the apparent K_m of polymerization, it binds primarily purines. It is possible that this site is not the same as the polymerization site observed by kinetic studies. It is also recognized that since the DNA template is not present in the binding studies and its influence on NTP binding is likely to be profound, the interpretation of the binding data is necessarily limited. Kinetic arguments against the presence of an allosteric site have been presented (Anthony *et al.*, 1969a).

One interesting implication of this work is that the preferential initiation of RNA synthesis by purine NTP's is due at least partially to an initiation site on the enzyme.

References

Anthony, D. D., Wu, C. W., and Goldthwait, D. A. (1969a), *Biochemistry 8*, 246.

Anthony, D. D., Zeszotek, E., and Goldthwait, D. A. (1969b), *Biochim. Biophys. Acta 174*, 458.

Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. K. F. (1969), *Nature 221*, 43.

Craig, L. C., and King, T. P. (1962), *Methods Biochem. Anal. 10*, 175.

Fox, F., Robinson, W. S., Haselkorn, R., and Weiss, S. B. (1964), *J. Biol. Chem. 239*, 186.

Furth, J. J., Hurwitz, J., and Anders, M. (1962), *J. Biol. Chem. 237*, 2611.

Kornberg, A. (1969), *Science 163*, 1410.

Krakow, J. S. (1968), *Biochim. Biophys. Acta 166*, 459.

Maitra, U., and Hurwitz, J. (1965), *Proc. Natl. Acad. Sci. U. S. 54*, 815.

Maitra, U., and Hurwitz, J. (1967), *J. Biol. Chem. 242*, 4897.

Maitra, U., Nakata, Y., and Hurwitz, J. (1967), *J. Biol. Chem. 242*, 4908.

Randerath, K., and Randerath, E. (1967), *Methods Enzymol. 12a*, 323.

Scatchard, C. T. (1949), *Ann. N. Y. Acad. Sci. 51*, 660.

Sillen, L. G., and Martell, A. E. (1964), Stability Cons ants, London, The Chemical Society.

Sippel, A., and Hartmann, G. (1968), *Biochim. Biophys. Acta 157*, 218.

Smith, D. A., Ratliff, R. L., Trujillo, T. T., Williams, D. L., and Hayes, F. N. (1965), *J. Biol. Chem. 241*, 1915.

Velick, S. F., Parker, C. W., and Eisen, H. N. (1960), *Proc. Natl. Acad. Sci. U. S. 46*, 1470.

Wu, C. W., and Goldthwait, D. A. (1969), *Biochemistry 8*, 4450.

Yphantis, D. A. (1964), *Biochemistry 3*, 297.

Specificity in Self-Assembly of Bacteriophages Qβ and MS2*

C. M. Ling, P. P. Hung, and L. R. Overby

ABSTRACT: Using purified ribonucleic acid and protein components of two serologically and electrophoretically distinct phages Qβ and MS2, phage-like particles were readily assembled from homologous ribonucleic acid and protein as well as from heterologous components. The hybrid particles were antigenically and electrophoretically similar to the homologous particles and to the authentic phages having the same protein component.

The reassembled particles produced from all four ribonucleic acid and protein combinations had similar sedimentation properties in sucrose density gradient. They differed in that the hybrids had no or very low infectivity as compared with the homologous reassembled particles. Under conditions where two phage ribonucleic acids competed for one phage protein, the formation of homologous particles prevailed; whereas when two proteins competed for one ribonucleic acid, Qβ protein was predominantly incorporated into particles with either ribonucleic acid. Particles with mixed protein coat were not detected. Thus the interaction between protein subunits appeared to have absolute species specificity.

Reports from several laboratories show that the RNA coliphages fr (Hohn, 1967), MS2 (Sugiyama *et al.*, 1967), R17 (Roberts and Steitz, 1967), and Qβ (Hung and Overby, 1969) readily reassemble into phage-like particles from RNA and proteins isolated from the respective phages. Hohn (1969) has shown that the protein from fr phage can be induced to form particles by a variety of RNA species. Hiebert *et al.* (1968) found that protein from the plant virus CCMV also could be assembled around RNA from a variety of sources. Particles with mixed-protein coats have been assembled *in vitro* from components of different plant viruses (Wagner and Bancroft, 1968). These results suggest a minimum of specific

* From the Department of Molecular Biology, Abbott Laboratories, North Chicago, Illinois 60064. *Received May 20, 1969.*

requirements for the self-assembly interactions. The physically similar, but serologically different, coliphages Qβ and MS2 offer a convenient system for further studying relative affinities between viral RNA and protein. In the present studies lack of absolute specificity between RNA and protein was demonstrated in two-component self-assembly systems composed of Qβ RNA and MS2 protein, or *vice versa*. The heterologous components formed phage-like particles as readily as did the homologous components. However, competitive interactions involving one protein and two RNAs, gave more definitive results, and showed that there was a greater relative affinity between homologous RNA and protein than between the heterologous components.

Materials and Methods

Bacteria and Phages. Bacteriophages Qβ and MS2 were prepared in *Escherichia coli* Q13 cultures and purified as described previously (Overby *et al.*, 1966a).

Isolation and Purification of Phage RNA. Purified phage particles were dissociated into RNA and protein by sodium dodecyl sulfate and phenol extraction (Gierer and Schramm, 1956; Overby *et al.*, 1966b). The phenol phase containing the interface was reserved for protein isolation, below. Phage RNA was recovered from the aqueous phase by precipitation with ethanol in the presence of 0.2 M potassium acetate. The isolated RNA was further purified by column chromatography using an Agarose–DEAE-cellulose combination column in 0.01 M Tris buffer containing 1.2 M NaCl and 0.02 M $MgCl_2$ (Hung, 1969a). The RNA preparations thus obtained showed a $s_{20,w}$ of approximately 30 S and were biologically active in spheroplasts (Pace and Spiegelman, 1966). In reconstitution with phage proteins the RNA gave infectious particles (Hung and Overby, 1969). Radioactive phage RNA, labeled with ^3H or ^{32}P, was prepared from radioactive phages grown in cultures containing [^3H]uridine or [^{32}P]orthophosphate (Overby *et al.*, 1966a). An appropriate amount of carrier RNA was usually added to the radioactive RNA before purification. The yield of RNA was estimated by absorbance at 260 mμ using an $E_{1\,cm}^{1\%}$ of 250.

Isolation of Phage Proteins. The phenol phase from the phenol extraction mentioned above was further extracted twice with one-half volume of 0.01 M Tris buffer (pH 7.1) to remove trace amounts of RNA and the aqueous layers were discarded. Phage proteins were recovered from the phenol layer by precipitation at −20° with five volumes of methanol containing 3% sodium acetate. The collected precipitate was washed twice with cold methanol and dissolved in 5 M guanidine-HCl, plus 0.05 M mercaptoethanol in 0.1 M Tris-HCl buffer (pH 7.1). The protein solution was stored at −20° and used within 2 weeks. Radioactive proteins labeled with ^{14}C or ^3H were obtained from radioactive phages grown in cultures containing [^{14}C]amino acid mixtures or [^3H]tyrosine. Recovery of phage protein was estimated by lyophilizing and weighing an aliquot of the recovered material. It was generally about 90% of theoretical and about three times the amount of recovered RNA.

Reassembly of Phage Particles. The conditions for phage reassembly were essentially the same as reported previously (Hung and Overby, 1969). A typical 0.5-ml reaction mixture consisted of 500 μg of phage proteins, 125 μg of phage RNA, 2.5 mmoles of guanidine-HCl, and 25 μl of mercaptoethanol in 0.1 M Tris-HCl buffer (pH 7.1). The mixture was dialyzed against two changes of Tris-acetate buffer (0.1 M Tris, pH 7.2, 0.05 M KCl, and 0.02 M magnesium acetate) at 4° over a period of about 20 hr. The dialyzed mixtures were centrifuged at 10,000*g* to remove precipitates before further analyses.

Density Gradient Centrifugation. The reassembly reaction mixtures were analyzed by gradient centrifugation in 3–18% sucrose in Tris–EDTA buffer (0.01 M Tris, pH 7.1, and 0.005 M EDTA) with a Spinco SW-41 rotor at 40,000 rpm for 110 min at 4°. Fractions were collected from the bottom of the tube and analyzed for radioactivity, infectivity, or absorbancy at 260 mμ.

Infectivity Assay. Plaque-forming units (PFU) of the reassembled phage particles were usually determined before and after density gradient centrifugation using the standard two-layer agar technique (Adams, 1959; Overby *et al.*, 1966a). Specific antiviral rabbit sera were used to determine the serotypes of the reassembled phage particles.

Electrophoresis. Analyses were carried out in a LKB immunoelectrophoresis apparatus. Agarose plates were prepared by pouring 25 ml of a 0.8% solution of melted Agarose in 0.0125 M sodium phosphate buffer (pH 7.4) plus 0.005% sodium azide onto 2 × 10.5 in. glass plates. The same buffer was used in the electrode chambers. A sample (10 μg) was placed in each well, and electrophoresis was performed with 350 V and 10–15 mA/plate for 45–60 min at room temperature. The plates were then subjected to overnight double diffusion against diluted antisera (25 μl of 1:10 dilution) at 37°. The antigen–antibody precipitation bands were stained for 15 min with 0.1% brilliant blue in 5% acetic acid after soaking the Agarose plates in buffer for at least 5 hr and drying under warm air. When direct staining of the phage particles was desired, the plates were dried after the electrophoresis, and stained with the same dye. The stained Agarose plates were washed with 5% acetic acid and air dried. All the immunoelectrophoretic patterns were obtained with the crude reconstitution mixtures which had been cleared of precipitates by low-speed centrifugation. Under these experimental conditions, free phage proteins precipitated in the sample wells and did not form antigen–antibody precipitation bands, while free RNA migrated to the cathode and beyond the fastest moving nucleoprotein particles.

Immunogel Diffusion. The Agarose plates used for immunoelectrophoresis were also adapted for immunogel diffusion. Aliquots of either 10 μg of phage particles of 5 μl or specific antisera (1:10 dilution) were used in the wells. The samples were allowed to diffuse for 20 hr at 37°. The precipitation bands were stained as described above for immunoelectrophoresis.

Radioactivity Counting. Solutions containing radioactive phage particles were precipitated with trichloroacetic acid, filtered onto nitrocellulose filters, and counted in a scintillation spectrometer. The distribution of radioactivity on Agarose plates used for electrophoresis was estimated as follows. The Agarose slabs were sliced at 1-mm intervals, and each slice was heated in 1 ml of 0.01 M Tris-HCl buffer (pH 7.1) at 80° for 20 min to melt the Agarose. An aliquot of 5 ml of ice-cold 5% trichloroacetic acid was then added to each tube with vigorous mixing. They were then counted as usual after filtration onto nitrocellulose filters. The heating and vigorous mixing were essential for ^3H label because of high quenching by Agarose.

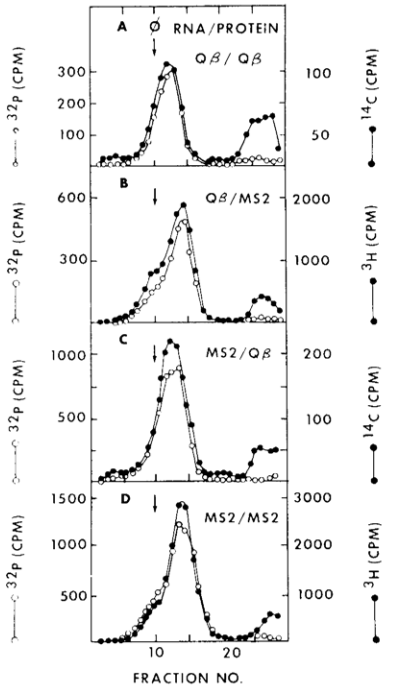

FIGURE 1: Sucrose density gradient centrifugation of reassembled particles containing labeled RNA and proteins. ^{32}P-Labeled Qβ RNA (0.2 mg) or MS2 RNA (0.2 mg) was reconstituted with ^{14}C-labeled Qβ protein (0.8 mg) or ^{3}H-labeled MS2 protein (0.8 mg) as described in Materials and Methods. The input radioactivities in cpm were: (A) ^{32}P, 3,200; ^{14}C, 1,800 (B) ^{32}P, 6,000; ^{3}H, 26,000; (C) ^{32}P, 11,000; ^{14}C, 3,200; (D) ^{32}P, 16,000; ^{3}H, 35,000. Reconstitution products were centrifuged in a 3–18% (w/v) linear sucrose density gradient at 4° for 110 min (40,000 rpm) in a Spinco SW41 rotor. Marker phages (φ), Qβ and MS2, were centrifuged in separate buckets. Fractions of 0.4 ml each were collected from the bottom (left in the figures) of each tube. One-half of each fraction was acid precipitated for radioactivity measurement.

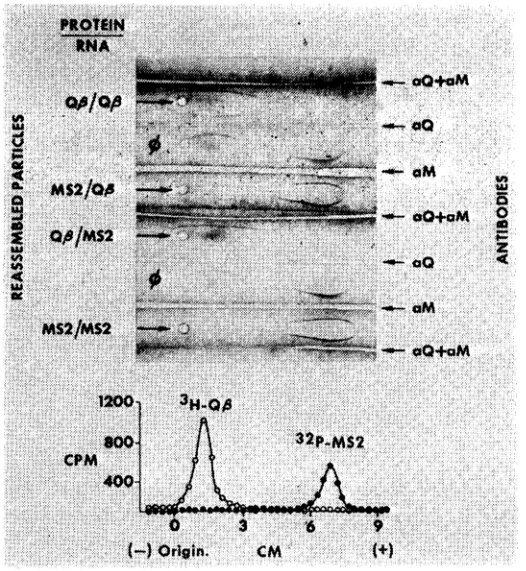

FIGURE 2: Immunoelectrophoreses of Qβ and MS2 phages and reassembled particles. Conditions for reassembly of the particles were the same as described in Figure 1. The radioactivity profiles were obtained after electrophoresis of [^{3}H]uridine-labeled Qβ and ^{32}P-labeled MS2 phages on the Agarose plate. φ indicates a mixture of equal amounts of Qβ and MS2 phages; aQ and aM indicate specific antibodies for Qβ and MS2, respectively (see Materials and Methods for experimental details).

Results

Physical and Biological Analyses of Homologous and Heterologous Reassembled Particles. ^{32}P-Labeled Qβ or MS2 RNA was reconstituted with ^{3}H-labeled MS2 protein or ^{14}C-labeled Qβ protein, using a protein to RNA mass ratio of 4. All four combinations of the two components were studied. Reassembled particles were observed in each case, and they appeared in a linear sucrose gradient as a main peak slightly lighter than authentic 80S phage particles (Figure 1). The sedimentation patterns of the particles reassembled from heterologous RNA and protein (Figure 1B,C) were indistinguishable from those reconstituted from homologous RNA and protein (Figure 1A,D). Authentic Qβ and MS2 are serologically distinct phages, and the protein and RNA differ in composition (Overby *et al.*, 1966b). Nevertheless, the protein and RNA from the two phages were clearly not species specific in regards to self-assembly into phage-like particles.

The reassembled particles were further analyzed for their electrophoretic properties by immunoelectrophoresis on Agarose plates (Figure 2). The immunoelectrophoretic patterns of the reassembled homologous particles closely resembled those of authentic phages. The heterologous particles electrophoresed like the phage from which the protein was derived. When labeled authentic phage particles were co-electrophoresed on the Agarose plates, the peaks of radioactivity coincided with the respective precipitation bands in the immunoelectrophoretic patterns (Figure 2). Similar results (not shown) were obtained with reassembled particles made from radioactive phage protein and RNA. These results established that the antigen–antibody precipitation bands observed in the immunoelectrophoretic patterns represented the actual location of the nucleoprotein particles in question.

In immunogel diffusion analyses, the reassembled particles were found to be serologically identical with the authentic phages (Figure 3). Anti-Qβ antibody formed precipitation bands only with authentic Qβ, reassembled Qβ, and reassembled particles made of Qβ protein and MS2 RNA; alternately, anti-MS2 antibody reacted only with authentic MS2, reassembled MS2, and reassembled particles made of MS2 protein and Qβ RNA. The continuity of the precipitation bands within each of the two groups indicated that all particles derived from the same phage protein were identical serologically.

The reassembled particles were analyzed for infectivity and, with the aid of specific antisera, for serotypes. Aliquots of 0.1 ml of the reconstitution mixture, or of a suitable dilution of it, were incubated with 0.1 ml of 1:100 diluted Qβ or MS2 antiserum at room temperature for 90 min before plating.

As shown in Table I, all the reassembled particles were completely inhibited by the antibodies specific for the phage that contributed the protein to the reassembled particles. The partial inhibition of the hybrids by the antibody corresponding to the RNA was probably the result of the high strength of antibodies used. This level of antiserum had low but measurably inhibitory effect by being transferred to the

TABLE 1: PFU Assay and Serotypes of Reassembled Particles.

Components		Antibody		PFU/RNA
Protein	RNA	Treatment	PFU/ml	Strand
Qβ	Qβ	None	2.3×10^{6}	3.0×10^{-8}
		Anti-Qβ	0	0
		Anti-MS2	2.1×10^{6}	2.8×10^{-8}
MS2	Qβ	None	1.6×10^{3}	2.1×10^{-11}
		Anti-Qβ	530	0.7×10^{-11}
		Anti-MS2	0	0
Qβ	MS2	None	210	2.8×10^{-12}
		Anti-Qβ	0	0
		Anti-MS2	60	0.8×10^{-12}
MS2	MS2	None	1.2×10^{7}	1.6×10^{-7}
		Anti-Qβ	1.1×10^{7}	1.5×10^{-7}
		Anti-MS2	0	0

agar plate and inhibiting the progenies, which in a phenotypic cross would have serotyes different from the parents. The result was commensurate with the idea of phenotypic crossing. It must be pointed out, however, that the specific infectivity (PFU/RNA strand) of the "hybrid particles" was less than 10^{-11} as compared with 10^{-7} in the homologous systems.

Relative Affinity of Two Phage RNAs for Individual Phage Proteins. When a mixture of MS2 and Qβ RNA was reassembled with a limited amount of either phage protein the relative amounts of each RNA encapsulated into particles gave a measure of the relative affinity between the RNA and each protein. The weight ratio of protein:RNA in mature phage particles is about 3:1 (Overby *et al.*, 1966a). Figure 4A shows the results of a reassembly experiment when the weight ratio of MS2 protein:Qβ RNA:MS2 RNA was 4 to 1 to 1. There was insufficient protein to fully encapsulate all of both RNAs. The incorporation of MS2 RNA into phage-like particles was three times as high as that of Qβ RNA. Under similar conditions (Figure 4B), Qβ protein encapsulated approximately 50% more Qβ RNA than MS2 RNA. The degree of affinity of the homologous RNA and protein varied among different preparations of phage proteins, and with the length of time that the proteins had been dissolved in 5 M guanidine–0.05 M mercaptoethanol–0.1 M Tris buffer (pH 7.1) and stored at −20°. Occasionally, the preference for Qβ RNA by Qβ protein was as high as 4 to 1. This species specificity appeared to be quite unstable and easily destroyed upon storage and by manipulations during the preparation of the protein from the phage. The species specificity of MS2 protein for MS2 RNA was generally better preserved; however, it also disappeared after long periods of storage in the presence of guanidine and mercaptoethanol. Preparations of Qβ protein and of MS2 protein which had been stored in guanidine–mercaptoethanol–Tris buffer at −20° for 2 months completely lost their species specificity without appreciable impairment of the ability to form phage-like particles (Figure 5).

Relative Affinity of Two Phage Proteins for Individual Phage RNA. Equal amounts of Qβ and MS2 proteins were allowed to reassemble with either of the two [^{32}P]phage RNAs. The

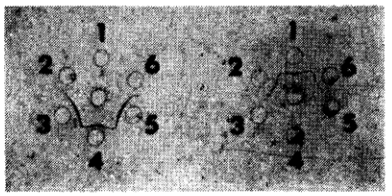

FIGURE 3: Immunogel diffusion of Qβ and MS2 phages and reassembled particles. (1) Qβ, (4) MS2; (2,3,5, and 6), reassembled particles made, respectively, from Qβ RNA/Qβ protein (2), MS2 RNA/MS2 protein (3), Qβ RNA/MS2 protein (5), and MS2 RNA/Qβ protein (6); left center well, MS2 antibody; right center well, Qβ antibody (see Materials and Methods for details).

products were analyzed by sedimentation in a linear sucrose gradient. At high total protein to RNA ratios (12:1 and 6:1), almost all the [^{32}P]RNA sedimented as the normal reassembled particles; whereas at lower protein:RNA ratios, a slow sedimenting peak of [^{32}P]RNA, similar in sedimentation to the free phage RNA, appeared in progressively increasing amounts at the expense of the peak of the phage-like particles. Thus, all of the RNA did not interact with insufficient amounts of protein and form a spectrum of partially finished nucleoproteins.

The above reconstitution products were further analyzed by immunoelectrophoresis to determine the distribution of the two proteins in the reassembled particles. As shown in Figure 6, the results depended upon the protein:RNA ratios. If MS2 RNA was reassembled with equal amounts of Qβ and MS2 proteins, and the total protein-to-RNA ratio was 2 or lower, there were two precipitation bands, equivalent to Qβ and MS2, respectively. There was only one band, equivalent to Qβ, at higher ratios. Direct staining of the plates after electrophoresis (without the double diffusion with antibodies) produced equivalent spots (Figure 6). Thus, the

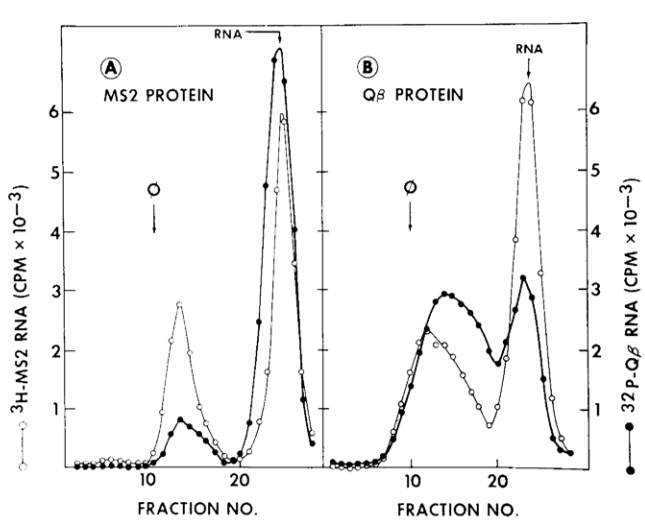

FIGURE 4: Sucrose density gradient centrifugation of particles reassembled from one phage protein and two phage RNA. Mixtures of [^{3}H]uridine–MS2 RNAs (0.125 mg; 60,000 cpm) and [^{32}P]Qβ RNA (0.125 mg; 60,000 cpm) were reconstituted with 0.5 mg of MS2 protein (A) or Qβ protein (B). Reconstitution products were subjected to sucrose density gradient centrifugation and fractions were collected and analyzed for radioactivity as described in Figure 1. Arrows indicate the peak position of phages and RNA, respectively.

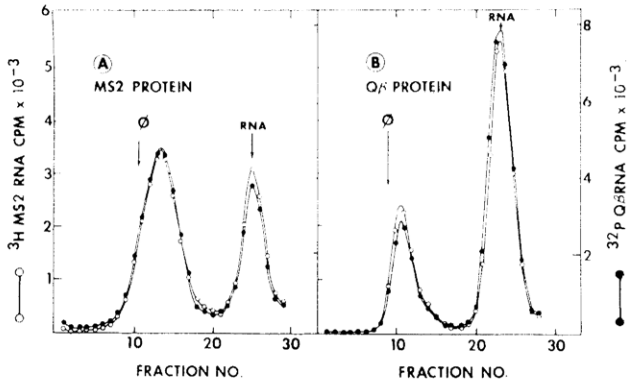

FIGURE 5: Sucrose density gradient centrifugation of particles reassembled from phage RNA and aged phage proteins. All experimental conditions were the same as Figure 4 except the protein preparations used in this experiment had been stored at −20° in 5 M guanidine 5% mercaptoethanol 0.1 M Tris (pH 7.1) for 2 months.

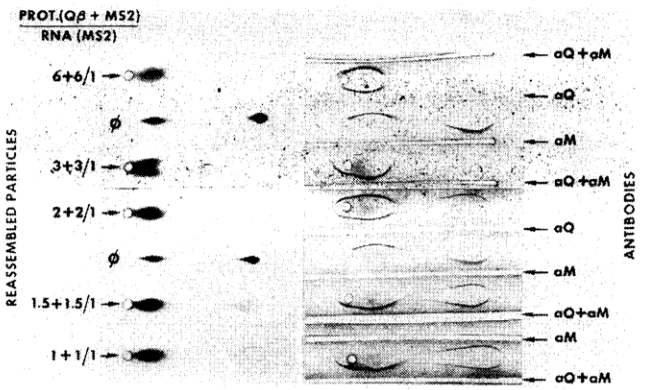

FIGURE 6: Immunoelectrophoretic and electrophoretic patterns of particles reassembled from MS2 RNA and two phage proteins. Mixtures of equal amounts of Qβ and MS2 proteins were reconstituted with MS2 RNA (0.1 mg) at the protein:RNA ratios indicated; *e.g.*, ratio 6 + 6/1 refers to six parts of Qβ protein and six parts of MS2 protein to one part of MS2 RNA on a weight basis. Final protein concentration in the reconstitution mixtures was 1 mg/ml. Other conditions were the same as in Figure 1. Right: immunoelectrophoresis; left: stained without double diffusion with antibodies. aQ, Qβ antibody; aM, MS2 antibody. (See Materials and Methods for details.)

stainable material and the material responsible for forming the antigen–antibody precipitation bands were identical. This observation indicates that the protein moiety of the individual particles was either Qβ or MS2, but not both. Thus Qβ and MS2 proteins do not cooperate to form phage-like particles.

At protein-to-RNA ratios greater than 2, Qβ protein was much more active than MS2 protein in encapsulating MS2 RNA. Only at low protein-to-RNA ratios, where Qβ protein alone was insufficient to encapsulate all the RNA, did MS2 protein assemble with MS2 RNA into MS2-like particles. The immunoelectrophoretic patterns of the particles reconstituted from Qβ RNA and the two phage proteins were quite similar to those with MS2 RNA as shown in Figure 6.

Discussion

In these self-assembly experiments there was no barrier for RNA and proteins from different phage origins to assemble into nucleoprotein particles. However, some preference by each protein for its homologous RNA did exist when two RNAs competed for one protein (Figure 4). The degree of preference observed in these cases should only be minimal, since the demonstratable species specificity was dependent upon the history of the protein preparations. The phage proteins were dissolved under reducing and denaturation conditions, and it is possible that this could impair their specificities without affecting their particle-forming properties. Another possibility was that the species specificities could involve a minor component in the phage protein preparations. We have shown earlier (Hung and Overby, 1969) that a minor protein component in the preparations from Qβ disappeared from solution upon storage in the presence of guanidine and mercaptoethanol. Particles assembled from such aged Qβ protein preparations and Qβ RNA showed no or very low infectivity. This could be due to loss of inactivation of a gene product, "A" protein (Lodish *et al.*, 1965; Argetsinger and Gussin, 1966; Roberts and Steitz, 1967; Steitz, 1968). This protein is required for infectivity of the phage. The lack of species specificity in the reassembly of particles from Qβ or MS2 RNA and aged Qβ or MS2 protein preparations (Figure 5) appeared to coincide with the absence of infectivity in the reassembled particles (Hung and Overby, 1969). It is, therefore, possible that the maturation protein ("A" protein) is also required for the expression of species specificity in the assembly of these phages. This question can be decided when adequate quantities of purified "A" protein and coat protein are available.

The absolute species specificity in the interaction among the phage protein molecules (Figure 6) is of interest. Particles with mixed protein coats have been assembled *in vitro* from components of different plant viruses (Wagner and Bancroft, 1968; Hiebert *et al.*, 1968). The complete segregation of the Qβ and MS2 proteins in the reassembled particles suggests the possibility that once the assembly was initiated by the attachment of a few protein units of one kind to an RNA molecule, this RNA molecule would be destinated to have that kind of coat protein (Hung, 1969b). The initial RNA–protein complex could therefore be regarded as an assembly center. However, the observations suggest that formation of a postulated assembly center was a rate-limiting process. In the reconstitution mixtures with low protein-to-RNA ratios the coat subunits were always in great excess of RNA on a molecular basis. At protein-to-RNA ratio of 2 on a weight basis, the ratio on molecular basis would be 120. Thus with a rapid initial interaction all the RNA molecules should form assembly centers. Consequently, all the RNA would be partially encapsulated to various degrees of completion when the protein component was exhausted. In the sedimentation patterns of the reassembly products (Figures 4 and 5) the two distinct peaks, one representing phage-like particles and the other equivalent to free RNA, would not have been observed. Thus, the assembly of particles could involve two kinetic processes: a slow and rate-limiting process for the formation of the initiation centers, followed by a much

faster cooperative process for the completion of the assembly into particles. As soon as the RNA formed a RNA–protein complex with a small number of protein molecules, this complex would continue to attract more molecules in an accelerated rate to form phage-like particles. Nonparticle RNA due to the exhaustion of the protein, would show a sedimentation pattern like free RNA.

The apparent greater affinity of Qβ protein for either Qβ or MS2 RNA, than was the case for MS2 protein, suggested that Qβ protein formed assembly centers with either RNA more readily than MS2 protein. Consequently, at low protein-to-RNA ratios, the assembly centers formed with Qβ protein were more than the amount of Qβ protein available to make complete particles. The final product, being deficient in protein, appeared to be more heterogeneous in the sedimentation patterns (Figure 4B).

Based on the findings discussed above, the chemical natures of the proteins and RNA of these two phages do not confer absolute species specificity in the assembly of phage particles. Under conditions where two phage RNAs and two phage proteins can freely interact, the products of self-assembly would be Qβ, MS2, and two hybrids with each protein and each RNA. No mixed-coat particles should form. Experiments are underway to determine the products formed *in vivo* when cells are dually infected with the two phages used in these self-assembly studies.

Acknowledgments

We wish to acknowledge the expert technical assistance of L. Straube and D. S. Jones and of W. Schulze. The authors are grateful to Dr. H. Wm. Sievert, Jr., for his valuable advice in immunoelectrophoresis.

References

Adams, M. H. (1959), *in* Bacteriophages, New York, N. Y., Interscience, p 111.

Argetsinger, J. E., and Gussin, G. N. (1966), *J. Mol. Biol. 21*, 421.

Gierer, A., and Schramm, G. (1956), *Z. Naturforsch. 11b*, 138.

Hiebert, E., Bancroft, J. B., and Bracker, C. E. (1968), *Virology 34*, 492.

Hohn, T. (1967), *European J. Biochem. 2*, 152.

Hohn, T. (1969), *J. Mol. Biol. 43*, 191.

Hung, P. P. (1969a), *Biochim. Biophys. Acta 186*, 220.

Hung, P. P. (1969b), *Federation Proc. 28*, 732.

Hung, P. P., and Overby, L. R. (1969), *Biochemistry 8*, 820.

Lodish, H. F., Horiuchi, K., and Zinder, N. D. (1965), *Virology 27*, 139.

Overby, L. R., Barlow, G. H., Doi, R. H., Jacob, M., and Spiegelman, S. (1966a), *J. Bacteriol. 91*, 442.

Overby, L. R., Barlow, G. H., Doi, R. H., Jacob, M., and Spiegelman, S. (1966b), *J. Bacteriol. 92*, 739.

Pace, N. R., and Spiegelman, S. (1966), *Proc. Natl. Acad. Sci. U. S. 55*, 1608.

Roberts, J. W., and Steitz, J. E. A. (1967), *Proc. Natl. Acad. Sci. U. S. 58*, 1416.

Steitz, J. A. (1968), *J. Mol. Biol. 33*, 937.

Sugiyama, T., Hebert, R. R., and Hartman, K. A. (1967), *J. Mol. Biol. 25*, 455.

Wagner, G. W., and Bancroft, J. B. (1968), *Virology 34*, 748.