The Reconstitution of Infective Bacteriophage Qβ*

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ABSTRACT: Infective and noninfective particles were reconstituted *in vitro* from purified ribonucleic acid, coat protein, and a minor protein of the bacteriophage Qβ. These individual components contained no infectious units but the ribonucleic acid was infectious in spheroplasts. Noninfective particles appeared to have similar size, shape, antigenicity, and ultraviolet absorption spectra as authentic phage, but differed in sedimentation coefficient and ribonuclease resistance. Formation of particles was related to input coat protein to ribonucleic acid ratio. The average sedimentation coefficient of the ribonucleic acid—protein complex increased from 30 to 70 S with increasing ratios of coat protein to ribonucleic acid. Infectivity of reconstituted particles was dependent upon the presence of the minor protein component in the reaction mixture. A linear response of infectivity was observed with increasing amount of this component with an optimal coat protein to ribonucleic acid ratio. Reconstituted infective particles sedimented as authentic Qβ phage in sucrose gradients, while noninfective particles, deficient in coat protein but containing the minor protein, sedimented lighter than the infective particles. Thus, reconstitution of infective Qβ particle *in vitro* requires all of the following conditions: (1) biologically active RNA, (2) sufficient coat protein to yield 80S particles, and (3) incorporation of another minor protein into the 80S particles.

Genetic and physical studies (Lodish *et al.*, 1965; Argetsinger and Gussin, 1966; Steitz, 1968) have indicated that infective RNA coliphages related to f-2 are composed of three components: a single strand of RNA, a structural coat protein, and an attachment protein ("A" protein). These components are assembled within host cell rather efficiently, yielding a population of phage in which about one out of five particles normally appears as a plaque-forming unit in agar-plating assays. When coat protein and RNA of fr (Hohn, 1967) or MS2 (Sugiyama *et al.*, 1967) were brought together *in vitro*, particles resembling the natural virus were readily formed. Inclusion of the "A" protein component in a reconstitution mixture of R17 resulted in significant increase in infective particles (Roberts and Steitz, 1967). There is as yet no genetic evidence for the presence of an "A" protein component in Qβ phage. In studies with this physically similar but serologically unrelated phage we observed that self-assembly also requires RNA, coat protein, and another protein component which will be referred here as minor protein. Levels of infectivity of reassembled particles were directly related to the amounts of the latter protein in the reaction mixture. Moreover, incorporation of this protein into particles which were deficient in coat protein did not produce infective particles. Detailed analyses of reassembly conditions provided some insight into the requirements for production of infectious particles. Self-assembly of polyhedral plant virus from its components has recently been reported. Hiebert *et al.* (1968) found that CCMV protein could be reassembled around RNA from a variety of sources to give in some instances infectious nucleoprotein particles.

Materials and Methods

Bacterium and Phage. Bacteriophage Qβ was prepared in *Escherichia coli* Q13 cultures and purified as described previously (Overby *et al.*, 1966a).

Isolation and Purification of Qβ RNA. Phenol extraction (Gierer and Schramm, 1956) was used to obtain crude RNA. It was further purified with DEAE-cellulose chromatography with a 0.3–1.2 M NaCl gradient elution (P. Hung and L. Overby, 1968, manuscript in preparation). As shown in Figure 1, RNA preparations thus obtained were biologically active in spheroplasts (Pace and Spiegelman, 1966). It was free from phage contamination and therefore suitable to be used for reconstituting infective particles. Radioactive Qβ RNA labeled with ^{3}H or ^{32}P was obtained from radioactive phage grown in cultures containing [^{3}H]uridine or [^{32}P]P_i (Overby *et al.*, 1966b). The radioactive RNA was then diluted with appropriate amount of carrier RNA and purified by DEAE chromatography.

Isolation of Qβ Proteins. Phage proteins were obtained by one of the following methods, each giving nearly quantitative recovery.

PHENOL METHOD. One volume of cold phage suspension (10–20 mg/ml) was extracted three times with an equal volume of phenol at room temperature in the presence of sodium dodecyl sulfate (0.5%) and EDTA (1 mM). Interface solids were left in the phenol layer. Combined phenol extracts were back-extracted with a Tris-HCl buffer (0.1 M, pH 7.2, 1 mM EDTA). Proteins were then precipitated at 4° from phenol with five volumes of methanol, containing 3% sodium acetate, centrifuged, and dried.

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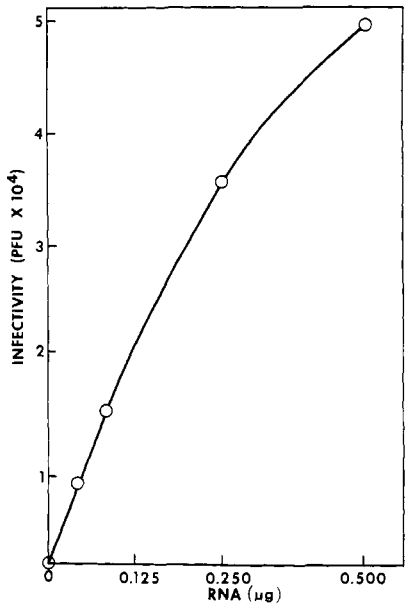

FIGURE 1: Infectivity of Qβ RNA in *E. coli* Q-13 spheroplasts (Pace and Spiegelman, 1966).

MERCAPTOETHANOL–GUANIDINE METHOD. Phage suspensions were treated with 5% mercaptoethanol and 3% guanidine hydrochloride for 2 hr at 4°. Dissociated proteins were separated from RNA by centrifuging on a 3–20% guanidine hydrochloride gradient (0.1 M Tris-HCl, pH 7.0, 1% mercaptoethanol) at 40,000 rpm for 3 hr in a Spinco SW41 rotor. RNA sedimented to the bottom half of the tube. Protein fractions near the top of the tube were precipitated with five volumes of cold ethanol and collected by centrifugation.

Preparation and Analysis of Radioactive Proteins. Phage was labeled by growing in media containing [^{3}H]tyrosine or [^{35}S]methionine. The phage thus prepared and purified had specific activities of 6×10^6 cpm/mg in ^{3}H and 2×10^6 cpm/mg in ^{35}S. Trace amounts of these radioactive phage preparations were added to a few milligrams of unlabeled phage and the proteins were isolated. Polyacrylamide gel electrophoresis of the labeled proteins was carried out by the method of Viñuela *et al.* (1967). Protein samples in 200 μl of 0.1% sodium dodecyl sulfate, 1% mercaptoethanol, and 8 M urea were applied to the gel after standing at room temperature for 20 min. Electrophoresis was performed for 210 min with 13 mA/tube at room temperature. Our original analysis of total Qβ phage protein (Overby *et al.*, 1966b) gave an *estimate* of 1 methionine residue and 4 tyrosine residues. The actual analytical values were 0.3–4. Subsequent analyses of purified coat protein and reexamination of the original results have indicated that the purified coat subunit lacks methionine. Recent analyses of the total extractable protein from purified phage show the presence of methionine but the tyrosine:methionine residue estimate is closer to 4:0.1 instead of the original estimate of 4:1. Polacrylamide electrophoresis of [^{3}H]tyrosine- or [^{35}S]methionine-labeled protein (Figure 2) supports these analyses, and shows the presence of at least two protein components in purified Qβ phage. Partial separation of the proteins was obtained by centrifuging mixtures ([^{3}H]tyrosine:[^{35}S]methionine ratio of about 4) in a guanidine hydrochloride gradient (3–20%) for 66 hr. Enrichment in the ^{35}S-labeled component in fractions along the tubes, calculated from changes in ^{3}H:^{35}S ratio, was at least tenfold. Detailed procedures for the specific labeling and the properties of the proteins will be published elsewhere. The ^{35}S minor protein was readily lost from solution during dialysis and subsequent storage presumably due to low concentration, and binding to surfaces. ^{3}H coat protein remained in solution during this period. Preparations lacking the minor protein were obtained by dialyzing a protein mixture from 8 M guanidine hydrochloride into the Tris-acetate buffer described below, and then "ageing" the solution at 4° for 1 month.

Reconstitution Condition. In a typical experiment, reaction mixtures contained the following components: 50 μg of biologically active Qβ RNA (1 mg/ml in 0.01 M Tris buffer, pH 7.2, plus 1 mM EDTA), 400 μg of Qβ protein (4 mg/ml in 0.1 M Tris (pH 7.2) plus 8 M guanidine hydrochloride and 1% mercaptoethanol), and minor protein concentrate (in 8 M guanidine hydrochloride with 1% mercaptoethanol) if used. Guanidine hydrochloride (8 M) was added to make a total volume of 500 μl. The mixtures were placed in dialysis sacs (five-times boiled) and dialyzed against two changes of a Tris-acetate buffer, TA buffer (0.1 M Tris (pH 7.2)–0.05 M KCl–0.02 M magnesium acetate), at 4°.

Analyses of Reconstitution Products by Ultracentrifugation. Reaction mixtures were analyzed by gradient centrifugation in 3–17% sucrose in TA buffer with a Spinco SW41 rotor at 40,000 rpm for 110 min at 4°. Fractions were collected and analyzed for radioactivity, infectivity, or absorbancy at 260 mμ. Nuclease sensitivity of labeled RNA in reconstituted particles was carried out by measuring acid-insoluble radioactivity before and after treatment with ribonuclease (Sigma five-times recrystallized, 5 μg/ml, 37° for 30 min in TA buffer). The effect of ribonuclease on infectivity of reconstituted particles was studied by treating the reaction mixtures with ribonuclease, followed by sucrose density gradient centrifugation and assaying collected fractions for infectious units. Reaction mixtures were also banded in cesium chloride in TA buffer with Spinco SW41 rotor for 66 hr at 40,000 rpm. The collected fractions were then analyzed for radioactivity or infectivity.

Electron Microscopy. Uranyl acetate staining of the sample was carried out according to Huxley and Zubay (1961). Phosphotungstic acid staining was done according to Brenner and Horne (1959). The specimen was examined with an RCA Model EMU 3G electron microscope.

Gel Filtration with Agarose. Reconstituted particles (1 A_{260} unit) purified by sucrose gradient centrifugation were combined with marker Qβ phage labeled with ^{32}P. The sample was placed on a column (0.9 × 55 cm) containing Bio-Gel A15M and was eluted with TA buffer. Radioactivity and A_{260} absorption were determined on eluted fractions to locate the position of marker phage and reconstituted particles.

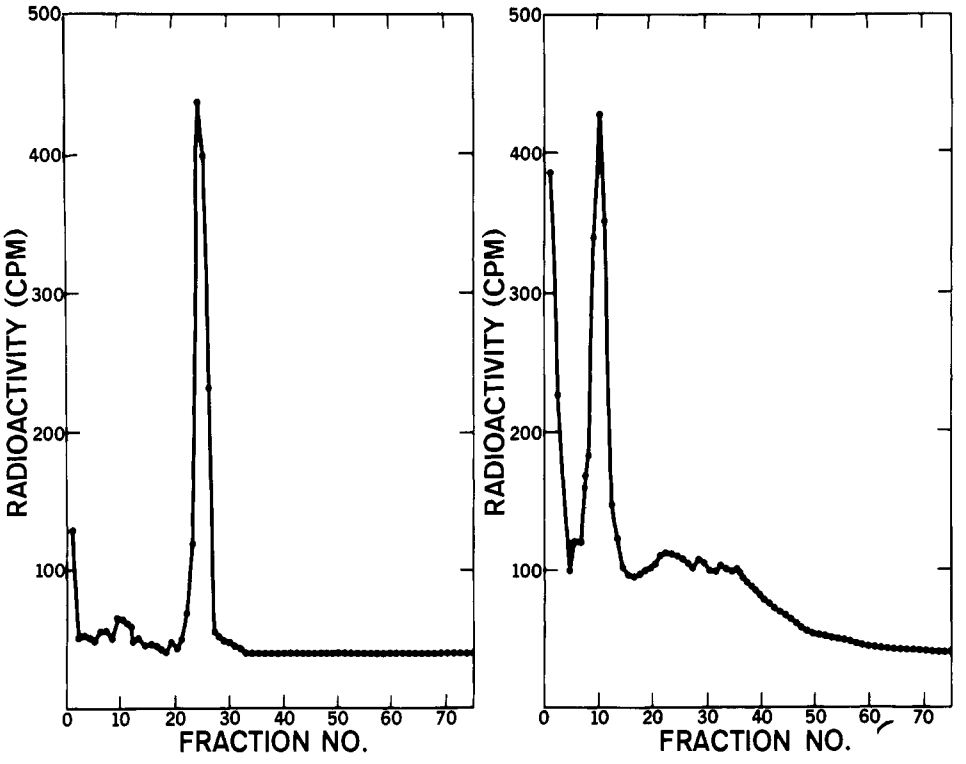

FIGURE 2: Polyacrylamide gel electrophoreses of Qβ proteins. Electrophoresis in the presence of 0.1% sodium dodecyl sulfate was performed as described in Materials and Methods. Left: Qβ protein labeled with [^{3}H]tyrosine (2000 cpm). After electrophoresis the gel was frozen and sliced into 1.25-mm disks for the extraction of radioactive material with 0.5 ml of TA buffer in a shaker overnight. The extracts were mixed with a counting solution (Kinard, 1957) for radioactivity measurements. The sample was electrophoresed from left (cathode) to right (anode). The radioactivity which remained at the origin was 47 cpm. Right: Qβ protein labeled with [^{35}S]methionine (5000 cpm) was electrophoresed and analyzed as described above. The radioactivity at the origin after electrophoresis was 58 cpm.

Ultraviolet Absorption. Reconstituted particles were purified by sucrose density gradient centrifugation and dialyzed into TA buffer. The preparations were then compared with authentic Qβ phage of the same concentration in a recording spectrophotometer.

Antigenicity of Reconstituted Particles. Competition between reconstituted particles and Qβ phage for anti-Qβ serum was tested in reaction mixtures containing the following: 1×10^9 plaque-forming units of Qβ phage; 100 μl of 1:80,000 diluted hyperimmune anti-Qβ serum; 0, 5×10^9, 1×10^{10}, or 1×10^{11} reconstituted particles; and normal saline to a final volume of 1 ml. The number of particles was estimated from the A_{260} value, where 1 A_{260} unit was 2×10^{13} particles. After 90 min at 37° the reaction mixtures were diluted and plated for measurement of remaining plaque-forming units.

Results

Size and Shape of Reconstituted Particles. Electron micrographs (Plate I) showed that reassembled particles were isometric and approximately the same dimension as Qβ phage. Generally, large and distorted particles were more frequent in reconstituted preparations than in normal phage samples. However, agarose column chromatography indicated that reconstituted particles had over-all dimensions similar to Qβ phage. Both reconstituted particles and [^{32}P]Qβ phage emerged from the column in the same region (Figure 3).

Ultraviolet Absorption. Ultraviolet absorption spectra of reconstituted particles, after purification with sucrose gradient centrifugation, and authentic phage are compared in Figure 4. The two spectra are similar, but the ratios of maximum and minimum absorption are considerably different. The ratio for purified Qβ phage was 1.59 and that for reconstituted particles, 1.31.

Density Gradient Centrifugation of Reconstituted Particles. Tritium-labeled Qβ RNA was reconstituted with Qβ protein (phenol method) at a protein to RNA ratio of 8. Centrifugation of the products in a linear sucrose gradient showed a single main peak, sedimenting lighter than the marker [^{32}P]Qβ (Figure 5). Analytical ultracentrifugation showed the average sedimentation coefficient of the reconstituted particles to be 70 S. The conversion of RNA into reconstituted particles varied from 20 to 50% under the reaction conditions used. The RNA in the purified reconstituted particles, recovered by phenol extraction, was infective in spheroplasts, but the efficiency of infection decreased from the original value of 4×10^{-7} plaque-forming unit/RNA molecule to 2×10^{-10}.

Ribonuclease treatment of reconstituted particles on individual tubes from the sucrose gradient centrifugation showed that about 50% of radioactivity from [^{3}H]RNA-labeled particles could be recovered as acid-

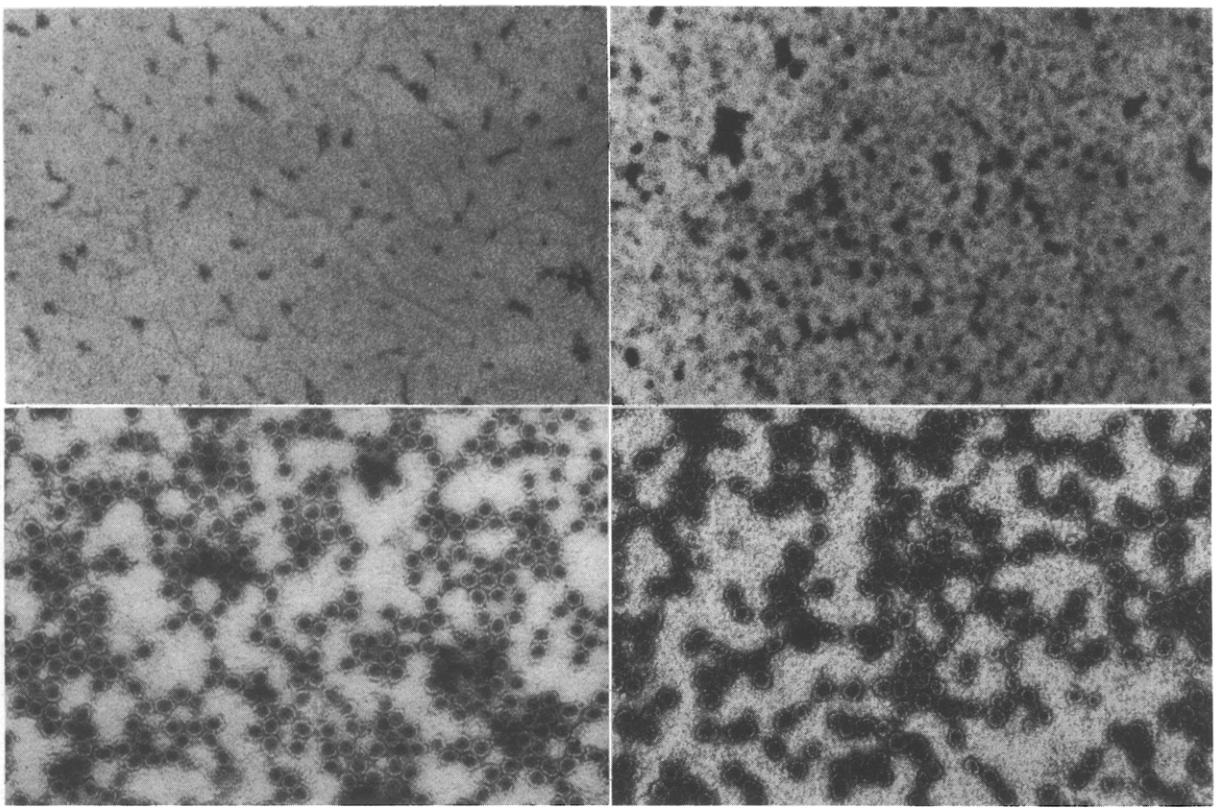

PLATE 1: Electron micrographs of Qβ RNA, Qβ coat protein, Qβ phage, and reconstituted particles, all magnified 150,000 times. Upper left: Qβ RNA in TA buffer with uranyl acetate stain. Upper right: Qβ coat protein in TA buffer with phosphotungstic acid stain; the protein was prepared by dissolving 4 mg in 1 ml of 8 M urea plus 10% mercaptoethanol and dialyzing against TA buffer. Lower left: Qβ phage stained with uranyl acetate. Average diameter of phage was 25 mμ. Lower right: reconstituted particles in TA buffer stained with uranyl acetate.

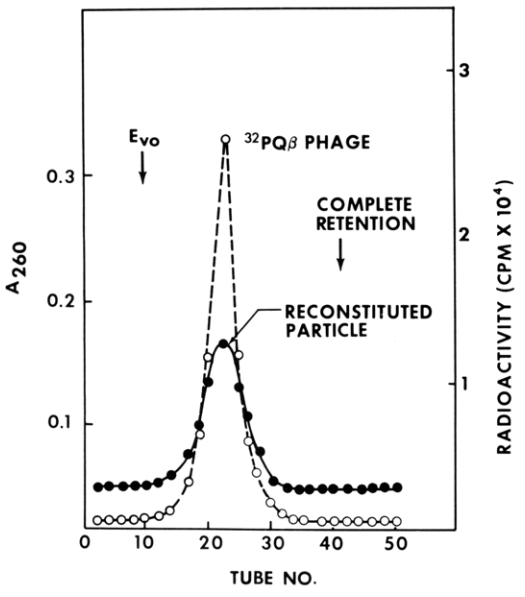

FIGURE 3: Gel filtration of reconstituted particles with Bio-Gel A15M. See Materials and Methods for details. Reconstituted particles are represented by solid circles and reference ^{32}P-labeled Qβ phage, by open circles.

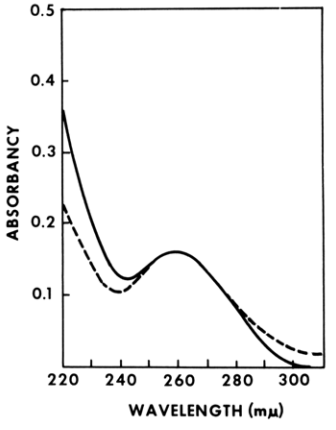

FIGURE 4: Ultraviolet absorption spectra of purified Qβ phage (dotted line) and reconstituted particles (solid line) in TA buffer.

insoluble counts after the treatment (Figure 5). In contrast, authentic Qβ phage labeled with ^{32}P was almost totally resistant to ribonuclease. In reaction mixtures containing 50 μg of RNA, 10^4–10^6 infectious units were generated. When marker phage was omitted

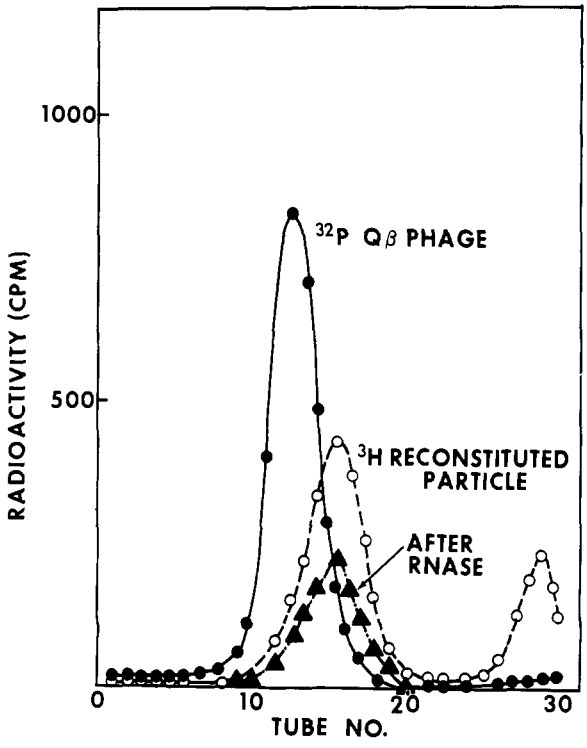

FIGURE 5: Sucrose density centrifugation of reconstituted particles containing labeled RNA. ^{3}H-labeled Qβ RNA (50 μg, 16,000 cpm) was reconstituted with Qβ protein (phenol method, 400 μg) as described in Materials and Methods. Reconstitution products were centrifuged with a marker [^{32}P]Qβ phage in a 3–17% (w/v) sucrose at 4° for 110 min (40,000 rpm) in Spinco SW41 rotor. Fractions of 0.4 ml each were collected from the bottom of the tube. Half of each fraction was acid precipitated for radioactivity measurement, and the other half first treated with ribonuclease (5 μg/ml, 37° for 30 min) and then counted for remaining acid-precipitable radioactivity. Solid circles: marker [^{32}P]Qβ phage. Open circles: [^{3}H]RNA in reconstituted particles. Solid triangles: [^{3}H]RNA in the particles after ribonuclease treatment.

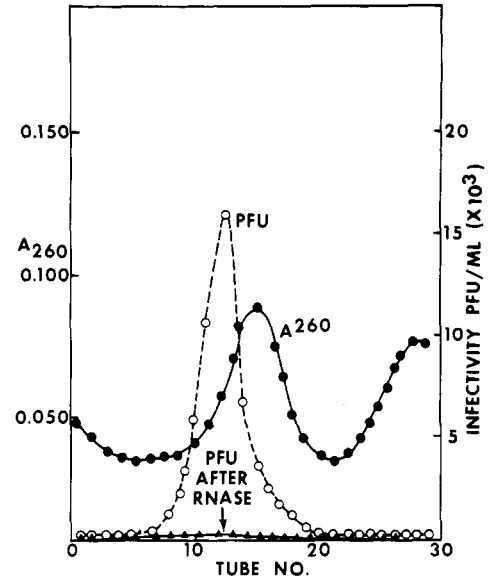

FIGURE 6: Sucrose density gradient centrifugation of infective reconstituted particles. Qβ RNA (50 μg) was reconstituted with Qβ protein (phenol method, 400 μg) into particles, giving approximately 4 × 10^4 plaque-forming units. Neither Qβ RNA nor protein showed any plaque-forming units by itself. The reconstituted mixture was divided into two portions. One was treated with ribonuclease (5 μg/ml) for 30 min at 37°. Both samples were then centrifuged in sucrose gradients as in Figure 5. Solid circles: absorbancy at 260 mμ of the untreated sample. Open circles: infectivity of the untreated sample. Triangles: infectivity of the sample treated with ribonuclease.

during gradient centrifugation, the infectivity peak of reconstituted particles was located at the 80S region (Figure 6). This did not match the bulk of the reconstituted particles. Ribonuclease treatment of reconstituted mixtures prior to gradient centrifugation eliminated more than 99% of the infectivity.

In another experiment 48% of [^{32}P]Qβ RNA was converted into 70S particles. When banded in cesium chloride about 60% of the reconstituted particles was stable (Figure 7). The radioactive peak was at a density of 1.43, indicating a protein to RNA ratio identical with Qβ phage. About 17% of the infectivity was recovered when infective reconstituted particles were banded in cesium chloride (Figure 8). Authentic Qβ phage tends to aggregate in cesium chloride solutions. However, both infective and physical particles are generally recoverable when low concentrations are banded.

Antigenicity of Reconstituted Particles. Serum-blocking capacity of reconstituted particles was tested in reaction mixtures containing constant amounts of anti-Qβ serum and infectious Qβ. The effect of increasing amounts of noninfective reconstituted particles is shown by a double-reciprocal plot in Figure 9. Reconstituted particles competed against Qβ phage for antiserum, and the slope of the plot revealed that in 90 min 20 molecules of reconstituted particles were required to neutralize antiserum equivalent to one plaque-forming phage. It is clear from extrapolation of the plot that an infinite number of reconstituted particles would neutralize all of the anti-Qβ serum; thereby all input Qβ phage would be recovered as plaque-forming units.

Effect of Initial Protein:RNA Ratio on S Value and Infectivity of Reconstituted Particles. Average sedimentation coefficients of reconstituted particles were found to be related to initial protein:RNA ratios. Sedimentation constants increased from the 30 S of free RNA to various intermediate values of nucleoprotein complexes and reached a plateau of 70 S with increasing ratios of protein to RNA (Figure 10). Although the natural ratio of protein to RNA found in Qβ phage is 3 on a weight basis, it takes about a ratio of 8 to reach the plateau under the reconstitution condition. Reconstituted particles, characterized by the average sedimentation constant in Figure 10, contained not only average particles but also heavier and lighter particles. Infective reconstituted particles were found only in the 80S region in sucrose gradient centrifugations (Figure 6). Therefore, the number of infective particles found in preparations with different protein/RNA ratios should be related to the population of 80S particles formed. As shown in Figure 10, infective

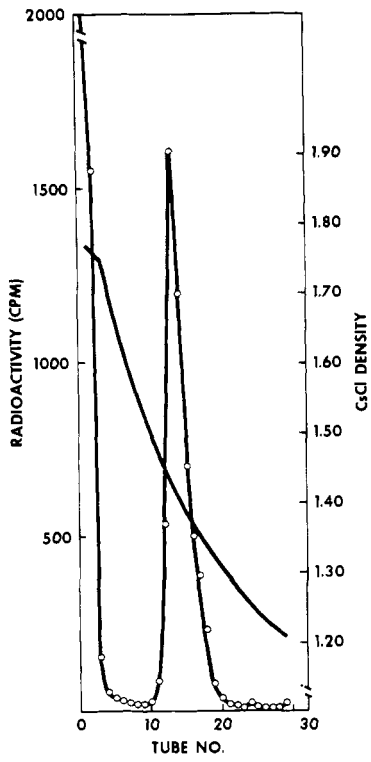

FIGURE 7: Cesium chloride density gradient centrifugation of reconstituted particles containing labeled RNA. ^{32}P-labeled Qβ RNA (1 mg, 1×10^5 cpm) was reconstituted with 8 mg of Qβ protein (by phenol method) as described in Materials and Methods. The mixture was centrifuged on a 3–17% (w/v) sucrose gradient as in Figure 5. The peak of reconstituted particles was collected and dialyzed into TA buffer. One milliliter of the purified particles (8×10^3 cpm) was combined with CsCl and TA buffer with average density of 1.43. The sample was centrifuged 66 hr at 40,000 rpm in Spinco SW41 rotor, and radioactivity of collected fractions was determined. Open circles: radioactivity of reconstituted particles; solid line: density gradient.

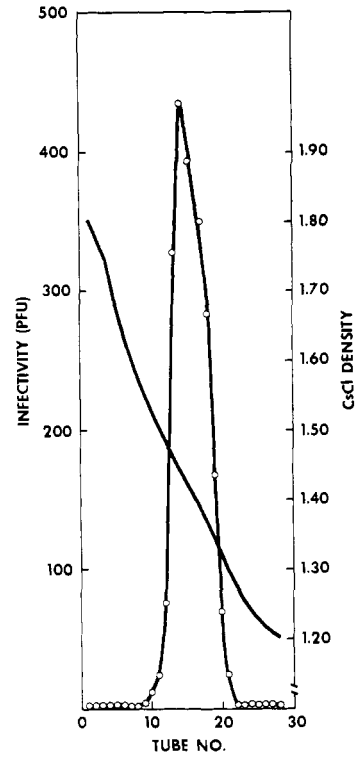

FIGURE 8: Cesium chloride density gradient centrifugation of infective reconstituted particles. Infective reconstituted particles (1.5×10^4 plaque-forming units) were centrifuged in CsCl as described in Figure 7 and remaining infective units assayed on collected fractions. Open circles: plaque-forming units per tube; solid line: density gradient.

particles and average sedimentation increased with higher protein:RNA ratio and reached a saturation level around the ratio of 8. Thus protein:RNA ratios affected not only average sedimentation constant, but also the level of infectivity. With aged protein preparations, which lacked the minor protein, particle formation was identical with that with the preparation containing both coat protein and the minor protein. However no infective particles were reconstituted with the aged protein preparation.

Dependence of Infectivity upon the Minor Protein. The above results suggested that the minor protein was necessary for infectivity. Various amounts of the minor protein preparation were added to reconstitution mixtures containing aged protein and Qβ RNA at the ratio of 8 where particle formation was not dependent upon additional coat protein. A linear response from 0 to 5×10^4 plaque-forming units per ml was observed as a function of the minor protein concentration in the reconstitution mixture (Figure 11). In contrast to this, fractions enriched in coat protein did not significantly stimulate the production of infectious units. In this experiment a plateau was not reached. When additional minor protein was added in subsequent experiments, the maximum yield of infective particles was 8×10^5 plaque-forming units/ml and the efficiency of conversion of RNA strands into infective particles was 3×10^{-8}.

Protein:RNA Ratios in Noninfective and Infective Reconstituted Particles. Protein preparations containing [^{3}H]tyrosine-labeled coat protein and [^{35}S]methionine-labeled minor protein were used to reconstitute particles with [^{32}P]Qβ RNA at a protein:RNA weight ratio of 5. The input ratio (^{32}P:^{35}S:^{3}H) of these three isotopes on the basis of cpm was 1:1.2:3.3. The natural ratio of the three isotopes in a Qβ phage mixture, which was constituted from separate preparations of [^{3}H]tyrosine, [^{35}S]methionine, and ^{32}P-labeled Qβ phage, was 1:0.74:1.93 for ^{32}P:^{35}S:^{3}H. Radioactivity distribution patterns of reconstituted particles after sucrose gradient centrifugation are shown in Figure 12. The ratio of the three isotopes at the 80S region where infectivity was located was 1:0.39:1.93. This indicated that the minor protein content was deficient, but coat protein content was similar to authentic phage. Light particles at the 65–70S region had a ratio of 1:0.26:1.47, indicating deficiencies in both the minor protein and coat protein.

Discussion

Icosahedral particles were readily assembled *in vitro* from the corresponding RNA and protein of Qβ phage.

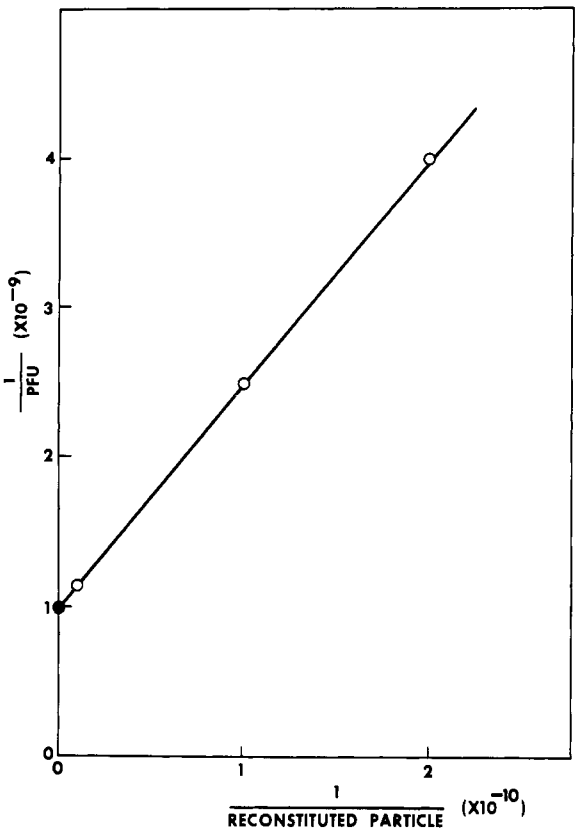

FIGURE 9: Double-reciprocal plot of competition for anti-Qβ serum between reconstituted particles and Qβ phage. See Materials and Methods for details. Reciprocals of the number of reconstituted particles were plotted against those of the remaining plaque-forming units (open circles) according to Lineweaver and Burk (1934). The solid circle at the ordinate is the control which contained only Qβ phage in the reaction mixture.

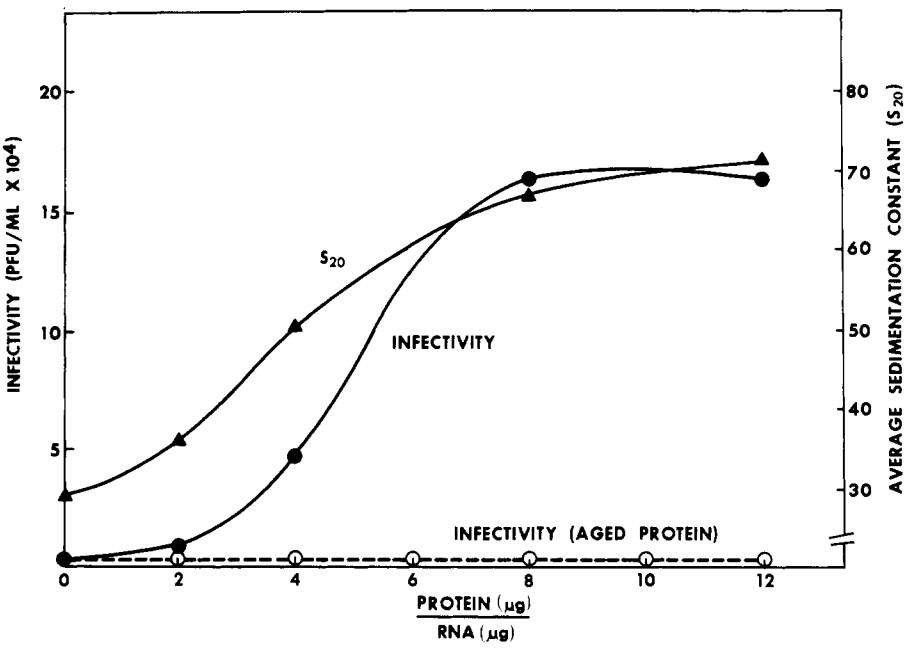

FIGURE 10: Effect of protein:RNA ratio on average sedimentation constant and infectivity of reconstituted particles. Reaction mixtures contained 50 μg of Qβ RNA (1 mg/ml) in Tris buffer (0.01 M, pH 7.2, 1 mM EDTA) and 0, 100, 200, 400, or 600 μg of Qβ protein (4 mg/ml, phenol method) in 8 M guanidine hydrochloride (0.1 M, Tris, pH 7.2; 1% mercaptoethanol). Average sedimentation constants of products formed after dialysis into TA buffer, as determined with Spinco Model E analytical ultracentrifuge, are shown in closed triangles. Infectivity of the reconstituted mixture is shown by solid circles. Open circles show infectivity of products when aged protein was used in a similar experiment. Sedimentation constants (not shown) of particles assembled from aged protein were very similar to those from fresh protein.

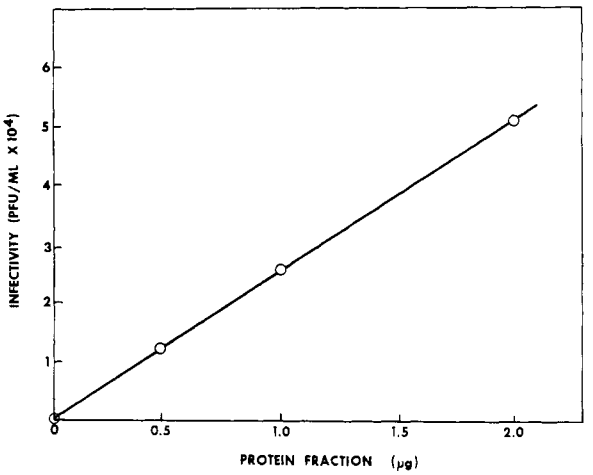

FIGURE 11: Dependence of infectivity of reconstituted particles upon the minor protein. Reaction mixtures contained 50 μg of Qβ RNA, 400 μg of aged Qβ protein (phenol method), and 0, 0.5, 1.0, or 2.0 μg of the minor protein concentrate preparation. After dialysis, the crude reaction mixtures were assayed for infective particles. Protein preparations enriched in (^{3}H) coat protein but deficient in [^{35}S]protein were used as controls. At the level of 2 μg, it enhanced the infectivity to 0.032×10^4 plaque-forming units/ml.

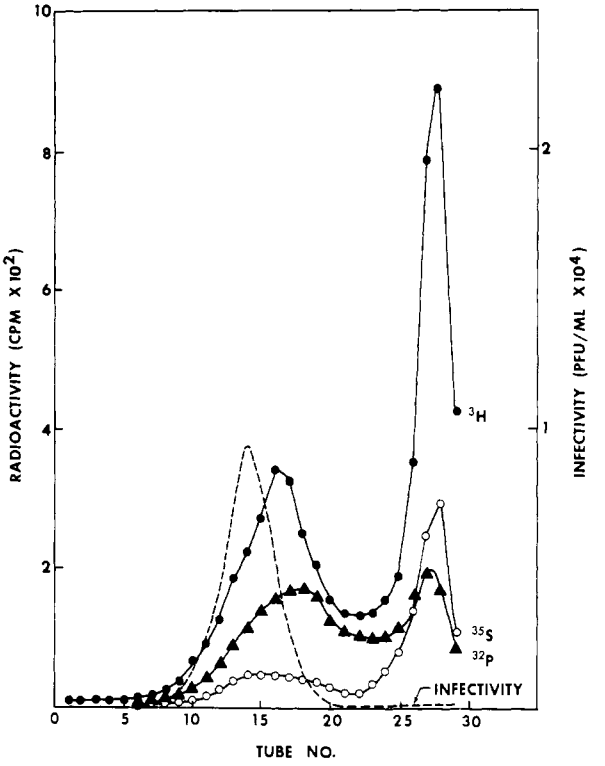

FIGURE 12: Incorporations of labeled coat protein, the minor protein, and Qβ RNA into reconstituted particles. The reconstitution reaction mixture contained 250 μg of [^{32}P]Qβ RNA (7140 cpm) and 1.25 mg of Qβ protein preparation (mercaptoethanol-guanidine method, ^{3}H coat protein, 23,500 cpm, and ^{35}S minor protein, 8650 cpm). After dialysis, an aliquot was centrifuged in a 3–17% (w/v) sucrose density gradient for 100 min in Spinco SW41 rotor at 40,000 rpm. Collected fractions were analyzed for their radioactivity and infectivity. Solid triangles: [^{32}P]Qβ RNA. Solid circles: ^{3}H coat protein. Open circles: ^{35}S minor protein. Dashed lines: infectivity.

Rigorously purified RNA and protein assured the absence of residual nucleoprotein susceptible to reactivation rather than reconstitution from individual components. In these experiments we have sought optimum conditions for reconstituting infective particles, identical with authentic phage. A large ratio of protein to RNA in the reconstitution mixture resulted in reassembled particles with high sedimentation coefficients. A high average sedimentation value resulted in increased infectivity. As shown in Figure 10, a protein to RNA ratio of 8 gave a product with an average value of 70 S and higher infectivity. Particles sedimenting lighter than 80 S appeared to be deficient in coat protein (Figure 12). These species predominated in all reconstitution reactions and were not infective despite the presence of the minor protein. In contrast to the lighter noninfective species, infective particles sedimented at the 80S region. These heavier particles possessed the full complement of coat protein, and a low but definite quantity of the minor protein. It appeared that reconstituted Qβ particles had to be fully coated to be infective. Reconstitution of infective particles also required another minor protein component for infectivity. This could be similar to the "A" protein of the R17 phage system (Roberts and Steitz, 1967). Particles were readily formed in the absence of the minor protein, and, at a protein:RNA ratio of 8, coat protein was not limiting for particle formation (Figure 10). As shown in Figure 11, infectivity showed a linear response to increasing amounts of the minor protein in reaction mixtures where particle formation was not dependent upon additional coat protein. Equal amounts of material containing coat protein only did not stimulate infectivity. These observations clearly indicated the requirements of the minor protein for infectivity. However, the mere presence of the minor protein did not assure infectivity. As shown in Figure 12, light-sedimenting particles contained the minor protein, but were not infective. Only a very few per cent of reassembled 80S particles were infective. There was sufficient minor protein, based on ^{35}S radioactivity, to account for about 10^{11} units of infectivity in tube 14 (Figure 12). However only about 10^4 infectious units were observed. Thus it appeared that 80S particles could be produced having the required content of coat protein and the minor protein, but still not infective. In this case the major limitation could be the intactness of the RNA, since icosahedral particles could be formed from noninfectious or even fragmented Qβ RNA (P. P. Hung and L. R. Overby, 1968, unpublished data). In the experiments reported here all the RNA started with was homogeneous 30S species and showed the maximum infectivity observable in spheroplasts: 10^{-5}–10^{-6} infectious unit/strand of RNA. Because of the inherent low efficiency of the spheroplast assay for infectious RNA, it is difficult to assess the actual potential infectivity of the RNA used. The reconstitution procedure itself could generate noninfectious RNA prior to com-

plete assembly into protected particles. Thus the recovery of infective reconstituted particles may depend upon the random association of an adequate number of coat protein molecules with one or a few minor protein molecule and one intact, infectious RNA molecule. Minimum availability or biological damage of one or all components could then indicate that infective combinations are rare events.

In contrast to the light but infective reconstituted particles (Roberts and Steitz, 1967), we have not observed significant infectivity in reconstituted light particles. Infective reconstituted particles closely resembled authentic phage in having the same density, sedimentation constant, and stability in cesium chloride. However they differed from authentic phage in that less than 1% survived ribonuclease treatment.

In contrast to the reconstitution of R17 particles where infectivity was enhanced by the addition of mercaptoethanol (1%) in the dialysis solution (Roberts and Steitz, 1967), we found a decrease in infectivity when the reaction mixture was dialyzed into TA buffer with 1% mercaptoethanol, or into the basic buffer of the R17 system. Inclusion of oxidized glutathione (0.005 M) in the dialysis liquid affected neither particle formation nor infectivity.

Purified reconstituted particles had antigenic determinants very similar to those of authentic phage. Although they competed with Qβ phage for anti-Qβ serum (Figure 9), their affinity was about eight times less than that of authentic phage.

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