Properties of the Ribonucleic Acid Bacteriophage ZIK/1 Coat Protein and its Synthesis in an *Escherichia coli* Cell-Free System

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The coat protein subunit of the RNA bacteriophage ZIK/1 has a molecular weight of 12 100 and does not contain histidine, methionine and cysteine. The amino acid composition of the coat protein is different from that of other RNA bacteriophage coat proteins. Bacteriophage ZIK/1 belongs to a class of RNA bacteriophages distinct from the f2 type, which lack histidine in their coat proteins, and the Q β type, which lack histidine and methionine. Bacteriophage ZIK/1 RNA is an efficient template in the *Escherichia coli* cell-free system producing coat protein as the major product and a number of non-coat proteins. This result is similar to that obtained with RNA from f2-type bacteriophages. It is probable that the genomes of RNA bacteriophages are structurally similar and that differences between the types of RNA bacteriophage arise from minor differences in RNA sequence.

RNA bacteriophages are morphologically similar and some of them have been shown to be serologically related (Bishop & Bradley, 1965; Scott, 1965). However, there are differences in the base compositions of their RNA (Table 1) and in the amino acid composition of the coat proteins (Table 2). It would appear from the G+C content of bacteriophage RNA that these bacteriophages can be divided into two groups: (a) ZIK/1, ZJ/1 and Q β ; (b) all others. In agreement with this, bacteriophage Q β coat protein differs markedly from those of RNA bacteriophages in group (b).

Analysis of RNA bacteriophage mutants has shown that there are three cistrons in the genome of bacteriophages f2 (Horiuchi *et al.*, 1966) and R17 (Gussin, 1966; Tooze & Weber, 1967). Three proteins have been identified by polyacrylamide-gel electrophoresis in actinomycin-treated *Escherichia coli* infected with bacteriophage MS2 (Nathans *et al.*, 1966; Vinuela *et al.*, 1967a, 1968); these proteins are the coat protein, RNA replicase and A-(maturation) protein. An additional A-protein has been detected in bacteriophage Q β -infected cells treated with actinomycin and in the intact virions (Garwes *et al.*, 1969), so it is possible that bacteriophage Q β may code for four proteins.

The RNA bacteriophage genome is an effective template in an *E. coli* cell-free system directing the synthesis of coat protein, which is the major product, and of non-coat proteins (Nathans *et al.*, 1962; Capecchi, 1966; Eggen *et al.*, 1967; Vinuela *et al.*, 1967b; Lodish & Robertson, 1969). The RNA replicase has been identified (Capecchi, 1966; Vinuela *et al.*, 1967b), and A-protein, which was

thought not to be synthesized in the cell-free system, has been shown to represent 1–2% of the total product (Lodish & Robertson, 1969).

It should be noted that studies on the translation of bacteriophage RNA in the *E. coli* cell-free system have all been carried out on bacteriophages of the f2 or higher-(G+C) group. The present paper describes the amino acid composition and molecular weight of bacteriophage ZIK/1 coat protein, and the translation of ZIK/1 RNA in a cell-free system.

Methods

Culture and purification of bacteriophage ZIK/1

Bacteriophage ZIK/1 and its host *E. coli* C3000/L were obtained from Dr. D. E. Bradley, Dept. of Zoology, University of Edinburgh. Bacteriophage inocula of high titre were prepared and assayed for plaque-forming units by the double-layer-agar plating technique as described by Adams (1959).

For large-scale cultures of bacteriophage ZIK/1 a modification of the procedure described by Bishop (1965) was used. The cultures were grown in 1.3% (w/v) nutrient broth (Oxo Ltd., London S.E.1, U.K.) containing 1 mM-CaCl₂, and unlysed bacteria and debris were removed by continuous-flow centrifugation at 10000g in a Griffin-Christ centrifuge. Bacteriophages were precipitated with (NH₄)₂SO₄ and purified by CsCl-density-gradient centrifugation.

[³H]Valine-labelled bacteriophages were prepared in a 200 ml culture in 1.3% (w/v) nutrient broth containing 1 mM-CaCl₂. When the concentration of bacteria reached 2 × 10⁸/ml 200 μ Ci of DL-[³H]valine

Table 1. *Base composition of bacteriophage RNA*

Bacteriophage	Composition (mol of G+C/100 mol)	Reference
Q β	48.4	Overby <i>et al.</i> (1966)
ZIK/1	48.1	Bishop & Bradley (1965)
ZJ/1	47.5	Bishop & Bradley (1965)
ZG	51.2	Bishop & Bradley (1965)
ZS/3	51.8	Bishop & Bradley (1965)
ZJ/3	51.8	Bishop & Bradley (1965)
α 15	51.3	Bishop & Bradley (1965)
MS2	52.0	Strauss & Sinsheimer (1963)
R17	51.9	Paranchych & Graham (1962)
f2	52.7	Loeb & Zinder (1961)
fr	52.0	Marvin & Hoffmann-Berling (1963)
β	52.6	Nonoyama <i>et al.</i> (1963)

(405 mCi/mmol; The Radiochemical Centre, Amer-sham, Bucks., U.K.) was added and 5 min later 4×10^{11} bacteriophages were added. After incubation for 3 h the culture was centrifuged at 12000g for 20 min to remove debris. The bacteriophages were pelleted by centrifugation at 100000g for 3 h and purified by CsCl-density-gradient centrifugation.

Isolation of bacteriophage ZIK/1 coat protein

Coat protein was extracted by degradation of purified bacteriophages with acetic acid in a manner similar to that described by Fraenkel-Conrat (1957). Acetic acid (2 vol.) was added dropwise to a cold suspension of bacteriophages (1–10 mg/ml) and the mixture was stirred for 30 min at 2°C. The precipitate was removed by centrifugation and the supernatant containing the coat protein was dialysed against several changes of 0.01 M-tris-HCl, pH 8.5, for 48 h at 2°C.

Extraction of bacteriophage ZIK/1 RNA

A portion (1 ml) of bacteriophage suspension (1–10 mg/ml) was mixed with 0.3 ml of 5% (w/v) sodium dodecyl sulphate for 5 min at room temperature and bentonite (10 mg), prepared by the method of Fraenkel-Conrat *et al.* (1961), was added. This mixture was extracted with phenol saturated with 0.1 M-tris-HCl, pH 7.2, and the RNA recovered by ethanol precipitation as described by Bishop (1965). RNA was stored at -20°C and assayed for infectivity by using protoplasts prepared from *E. coli* C3000/L as described by Strauss (1964).

Preparation of E. coli cell-free extracts

Extracts of *E. coli* A19 were prepared by a slight modification of the method of Nirenberg & Matthaei

(1961). Frozen cell paste (10 g) was ground with two parts of alumina (A305, bacteriological grade; Alcoa Chemicals Inc., Pittsburgh, Pa., U.S.A.) and extracted with 40 ml of 0.01 M-tris-HCl, pH 7.2, 0.01 M-magnesium acetate, 0.06 M-NH₄Cl, 6 mM-2-mercaptoethanol. The S30 supernatant was dialysed against several changes of the same buffer for 16 h at 2°C and stored in portions (1 ml) at -20°C. Protein concentration was measured by the method of Lowry *et al.* (1951) with bovine serum albumin [Sigma (London) Chemical Co., London S.W.6, U.K.] as the standard.

Measurement of polypeptide synthesis in E. coli cell-free extracts

Reactions were carried out at 37°C in volumes of 0.1–1.0 ml containing (per ml): 50 μ mol of tris-HCl (pH 8.1), 10 μ mol of magnesium acetate, 50 μ mol of NH₄Cl, 10 μ mol of GSH, 0.2 μ mol of GTP, 1.0 μ mol of ATP, 5.0 μ mol of phosphoenolpyruvate, 20 μ g of pyruvate kinase, 1–2 mg of S30 protein, 20–100 μ g of ZIK/1 RNA, 10–20 nmol of a ¹⁴C- or ³H-labelled amino acid and 20 nmol of each of 19 unlabelled amino acids. Incorporation was measured by spotting 10–50 μ l samples on 2 cm discs of Whatman 3MM filter paper. The discs were washed once with 5% (w/v) trichloroacetic acid, heated at 90°C for 30 min in 5% (w/v) trichloroacetic acid, washed twice more with acid, twice with ethanol-ether (2:1, v/v) and once with ether. Radioactivity was determined in a liquid-scintillation counter (Bishop, 1965).

For preparation of labelled polypeptides, the reaction was stopped after incubation at 37°C for 1 h by addition of an equal volume of 0.5 M-NaOH. The mixture was left at room temperature for 1 h and 1 vol. of 20% (w/v) trichloroacetic acid was added. After standing for 1 h in an ice bath the precipitate was collected by centrifugation and washed twice

with 5% (w/v) trichloroacetic acid, twice with ethanol-ether (2:1, v/v) and once with ether.

Polyacrylamide-gel electrophoresis

Purified polypeptides were dissolved in 0.1M- Na_2HPO_4 - NaH_2PO_4 buffer, pH 7.2, containing 1% (w/v) sodium dodecyl sulphate and dithiothreitol (1mg/ml) and incubated at 60°C for 1 h. Samples (50–100 μl) were mixed with 0.2 vol. of 2M-sucrose containing 0.01% Bromophenol Blue and applied to columns of polyacrylamide gel (8cm $\times$ 0.6cm) in Perspex tubes. The gels contained 7.5% (w/v) acrylamide, 0.2% NN' -methylenebisacrylamide, 0.1% ammonium persulphate, 0.08% (v/v) $NNN'N'$ -tetramethylethylenediamine, 0.1% sodium dodecyl sulphate, 0.1M- Na_2HPO_4 - NaH_2PO_4 , pH 7.2, and 1mM-EDTA. Electrophoresis was at 5V/cm for 3 h with a 0.1M-phosphate buffer, pH 7.2, containing 0.1% sodium dodecyl sulphate (under these conditions proteins migrate towards the anode). Gels were pre-run for 1 h before application of the sample.

To locate polypeptides the gels were fractionated into 1 mm slices with a razor blade and grooved block with transverse cuts at 1 mm intervals. Each slice was placed in a scintillation vial and left for 1 h with 0.4ml of 0.1% sodium dodecyl sulphate. To each vial 10ml of Kinard (1957) scintillation fluid was added and radioactivity was measured in a Tri-Carb liquid-scintillation spectrometer (Packard Instrument Co.).

Results

Properties of bacteriophage ZIK/1 coat protein

Determination of molecular weight. Coat protein was extracted from purified bacteriophages by using acetic acid as described in the Methods section and the molecular weight of the subunit was determined by the short-column sedimentation-equilibrium method (Yphantis, 1960). Freshly deionized 8M-urea was added to a solution of coat protein in 0.01M-tris-HCl buffer, pH 8.0 (5–10mg of protein/ml), and the solution was dialysed against 50ml of 0.01M-tris-HCl, pH 8.0, containing 6M-urea, for 16 h at 20°C with three changes of liquid. The protein solution was removed from the dialysis bag, diluted with buffer where necessary and samples were loaded immediately into a four-channel Yphantis cell; 21 μl of 0.01M-tris-HCl, pH 8.0–6M-urea was placed in each reference channel and 5 μl of FC43 oil (perfluorotriethylamine; Minnesota Mining and Manufacturing Co., St. Paul, Minn., U.S.A.) plus 15 μl of protein solution in each sample channel. The cell was centrifuged at 20000 rev./min or 30000 rev./min in a Spinco model E analytical ultracentrifuge. Determinations were carried out at 20°C by schlieren

optics with a constant phase-plate angle of 70°. Photographs were taken at hourly intervals and equilibrium was reached by 3 h. The apparent molecular weight of the coat-protein was calculated from the equation

$$M_{app.} = \frac{1}{rC_0} \left(\frac{dc}{dr} \right) \frac{RT}{\omega^2(1-\bar{v}\rho)}$$

where ω is angular velocity in rad/s, T is absolute temperature, R is the gas constant (8.314×10^7 erg/°K per mol), r is the distance of the mid-point of the column from the axis of rotation, C_0 is protein concentration, ρ is the density of the protein solution, (dc/dr) is the height of the column at the mid-point at equilibrium, and $\bar{v}$ is the partial specific volume of the protein.

The partial specific volume of bacteriophage ZIK/1 coat protein was calculated from the amino acid composition, as described by Cohn & Edsall (1943), and is equal to 0.737 cm³/g. The initial protein (C_0) was determined in terms of arbitrary units by integration of the synthetic-boundary trace obtained by centrifuging protein solution and buffer in a double-channel synthetic-boundary cell; the cell was centrifuged at 10000 rev./min and 20°C and photographs were taken after 1 h. The height of the column at the mid-point at equilibrium was measured directly from the difference between the solution and solvent traces in the same units as C_0 . This was done by enlarging the schlieren traces on to a photograph and measuring the distance between traces with a comparator.

Fig. 1. Relationship between protein concentration and apparent molecular weight determined by the Yphantis (1960) method

For experimental details see the text. Each point on the graph represents the mean of at least two determinations. The position of the line drawn through the points was determined by the method of least squares.

Measurements of the apparent molecular weight of bacteriophage ZIK/1 coat protein were made at several protein concentrations; Fig. 1 shows the relationship between molecular weight and initial protein concentration. The molecular weight appeared to be concentration-dependent and the real value was therefore measured at zero concentration from the intercept on the ordinate of the graph. This gave a value of 12100 ± 100 , which is less than that determined for other RNA-bacteriophage coat proteins. These have molecular weights of 14000–

15000, determined from the amino acid compositions shown in Table 2.

Amino acid composition. The amino acid composition of bacteriophage ZIK/1 coat protein was determined as described by Ambler & Brown (1967). Samples (2 mg) of coat protein were hydrolysed with 6M-HCl for 24–96 h and the hydrolysates analysed for amino acids by the method of Spackman *et al.* (1958). The results of these analyses are shown in Table 2. The coat-protein subunit contained 102 ± 2 amino acid residues and lacked histidine, methionine and

Table 2. *Amino acid composition of bacteriophage ZIK/1 coat protein*

Amino acid composition was determined as described by Ambler & Brown (1967). 'Best values' for serine and threonine are recoveries extrapolated to zero time of hydrolysis and, for valine and isoleucine, extrapolated to infinite time of hydrolysis. Best values for other amino acids are the means of the recoveries for the four hydrolysates. Values in residues/mol were calculated by dividing the best value for each amino acid by 0.0423. This number was obtained by dividing the sum of the best values for phenylalanine, tyrosine, lysine and arginine by 16, as these amino acids represent 16% of the total recovered and a protein of molecular weight 12000–13000 contains about 100 residues. Recoveries were corrected for possible inaccuracies in the weight of protein hydrolysed. The correction factor represents the ratio of the sum of the recoveries of lysine, arginine, aspartic acid, glutamic acid, alanine, phenylalanine and leucine to the sum of these amino acids in the 24 h hydrolysis sample. N.A., Not analysed.

Hydrolysis time (h)	Amount of amino acid recovered (μ mol)					Content (residues/mol)	Amino acid content of bacteriophage coat proteins (mol/mol of protein)		
...	24	24	48	96	Best value		f2†	MS2†	Qβ‡
Gly	0.310	0.319	0.319	0.313	0.315	7.4	9.9	10.1	7.8
Ala	0.524	0.533	0.526	0.529	0.528	12.5	15.0	15.0	14.7
Val	0.537	0.534	0.569	0.598	0.610	14.4	15.1	15.2	12.4
Leu	0.263	0.266	0.269	0.266	0.266	6.3	8.6	7.4	10.0
Ile	0.251	0.255	0.264	0.272	0.274	6.5	8.1	8.2	3.9
Ser	0.423	0.422	0.392	0.324	0.455	10.8	13.2	13.7	10.1
Thr	0.288	0.289	0.276	0.255	0.310	7.3	9.2	9.2	11.6
Asp	0.446	0.447	0.443	0.445	0.455	10.5	14.9	15.3	13.2
Glu	0.259	0.263	0.259	0.259	0.260	6.1	12.0	12.0	11.6
Phe	0.139	0.138	0.136	0.141	0.139	3.3	4.3	4.3	2.3
Tyr	0.163	0.164	0.159	0.159	0.161	3.8	4.1	4.1	3.1
Trp	—	—	—	—	—	0.5*	N.A.	2.0	0.0
Cys	—	—	—	—	—	0.0	N.A.	1.4	1.5
Met	—	—	—	—	—	0.0	1.0	2.0	0.8
Pro	0.161	0.145	0.148	0.178	0.157	3.7	6.3	6.4	7.0
Lys	0.216	0.217	0.220	0.218	0.218	5.2	6.0	6.0	7.8
His	—	—	—	—	—	0.0	0.0	0.0	0.0
Arg	0.164	0.152	0.161	0.158	0.159	3.8	4.3	4.4	7.0
NH ₃	0.385	0.393	0.552	0.519	0.325	7.7			
Correction factor	1.00	1.00	0.97	1.05					
Total	—	—	—	—	—	102.1	132.0	136.7	124.8

* Determined separately as described by Ambler & Brown (1967).

† Lin *et al.* (1967). The composition of the coat proteins of the bacteriophages R17, M12 (Enger & Kaesberg, 1965) and fr (Wittmann-Liebold, 1965) is almost identical with that of bacteriophage MS2 coat protein.

‡ Overby *et al.* (1966), based on a molecular weight of 15500.

cysteine. The amino acid composition of bacteriophage ZIK/1 coat protein is clearly different from those of the other RNA bacteriophages.

Polyacrylamide-gel electrophoresis of bacteriophage ZIK/1 coat protein

Purified coat protein or intact bacteriophages were disaggregated by incubation at 60°C in the presence of 1% (w/v) sodium dodecyl sulphate and subjected to electrophoresis on polyacrylamide gel as described in the Methods section. The protein was located by staining for 15 min with 1% (w/v) Amido Black in 10% (v/v) acetic acid; excess of dye was removed by soaking the gels in several changes of ethanol-acetic acid-water (4:1:5, by vol.), and the gels were scanned with a Chromoscan (Joyce-Loebl Ltd.) densitometer. One major band was obtained with purified coat protein or bacteriophages (Figs. 2a and 2b). Coat protein showed one or two very minor bands; the proportion of protein in these minor bands increased with storage of the protein before electrophoresis. Fig. 2(c) shows the electrophoretic profile obtained with protein that had been stored at 4°C for 4 weeks before disaggregation and electrophoresis. It has been shown that on polyacrylamide gel in the presence of sodium dodecyl sulphate the electrophoretic mobility of proteins is proportional to their molecular weight (Shapiro *et al.*, 1967). Comparison of the coat-protein bands with proteins of known molecular weight indicated that the molecular weight of the major band was 12 500 and those of the minor bands were 24 000 and 48 000. The molecular weight of the major monomer band agrees well with that determined by the sedimentation-equilibrium method, and those of the minor bands correspond to a coat-protein dimer and tetramer. The increase in the proportions of dimer and tetramer was probably caused by irreversible aggregate formation during storage.

Characteristics of protein synthesis in an E. coli cell-free system primed with bacteriophage ZIK/1 RNA

Amino acid incorporation directed by bacteriophage ZIK/1 RNA. The kinetics of incorporation of [¹⁴C]-leucine (165 mCi/mmol; The Radiochemical Centre) and [³H]histidine (9010 mCi/mmol; The Radiochemical Centre) in the presence of bacteriophage ZIK/1 RNA are shown in Fig. 3; incorporation of phenylalanine and valine had the same kinetics as that of leucine. Whereas leucine incorporation started immediately, there was a lag phase of 5–10 min in the incorporation of histidine, an amino acid that is absent from the coat protein. Thus synthesis of non-coat protein was not initiated directly and some translation of the coat cistron was required before initiation of non-coat-protein synthesis. A similar result has been obtained

Fig. 2. Densitometric traces of (a) purified ZIK/1 bacteriophages, (b) purified coat protein and (c) coat protein stored at 4°C for 4 weeks, after electrophoresis on polyacrylamide gels

Portions (100 µg) of coat protein were treated with sodium dodecyl sulphate and subjected to electrophoresis as described in the Methods section.

with RNA from bacteriophages MS2 (Ohtaka & Spiegelman, 1963) and f2 (Lodish, 1968).

Polyacrylamide-gel electrophoresis of proteins synthesized in the presence of bacteriophage ZIK/1 RNA,

Fig. 3. Amino acid incorporation in the *E. coli* cell-free system primed with bacteriophage ZIK/1 RNA

(a) Leucine incorporation, (b) histidine incorporation into acid-precipitable material in the presence (○) and absence (●) of RNA. Incorporation was measured in 10 μ l samples from 0.2 ml reaction mixtures as described in the Methods section.

Proteins labelled with [14 C]valine (135 mCi/mmol; The Radiochemical Centre) or with [3 H]histidine (9010 mCi/mmol) were isolated from a 0.2 ml reaction mixture and subjected to electrophoresis on polyacrylamide gels as described in the Methods section. [14 C]Valine-labelled proteins were mixed with 20 μ l of [3 H]valine-labelled purified coat protein (10800 c.p.m./ml) before treatment with sodium dodecyl sulphate and electrophoresis. Fig. 4 shows the electrophoretic profiles obtained with valine- and histidine-labelled proteins. No significant peaks were observed when RNA was absent from the cell-free system. The major product in the presence of bacteriophage ZIK/1 RNA was a large peak, which

Fig. 4. Polyacrylamide-gel electrophoresis of protein synthesized in the *E. coli* cell-free system in the presence of bacteriophage ZIK/1 RNA

(a) [3 H]Valine-labelled purified coat protein; (b) [14 C]valine-labelled proteins produced in the presence of bacteriophage ZIK/1 RNA mixed with purified coat protein (----); (c) [14 C]valine-labelled proteins produced in the absence of RNA; (d) [3 H]-histidine-labelled proteins produced in the presence and (e) in the absence of bacteriophage ZIK/1 RNA. For experimental details see the Methods section.

accounted for about 75% of the proteins synthesized and migrated with purified coat protein on electrophoresis. This peak, which was absent from the histidine-labelled proteins, was therefore identified as the coat protein. In addition to the large coat-protein peak, minor peaks were detected in the valine-labelled material (fractions 5–30 in Fig. 4b). Most of the histidine-labelled material migrated in this region and several peaks were observed (Fig. 4d). The difference in specific radioactivities and counting efficiencies of [^3H]histidine and [^{14}C]valine resulted in each histidine residue having approx. 30 times the radioactivity of each valine residue. Thus a protein containing ten valine residues and one of histidine would have about three times more radioactivity when labelled with histidine than with valine. It is therefore probable that all the minor peaks in the valine-labelled material are histidine-containing proteins. The peak of histidine-containing material (fractions 40–45, Fig. 4d) that migrated in front of the coat protein is probably equivalent to the shoulder on the leading edge of the coat-protein peak.

Analysis of proteins produced in an *E. coli* cell-free system primed with RNA from bacteriophages f2 or MS2 has shown that coat protein represents about 70% of the total product and that two or three well-defined minor peaks are detected on polyacrylamide-gel electrophoresis (Nathans *et al.*, 1966; Eggen *et al.*, 1967; Vinuela *et al.*, 1967b). One of these peaks, which represents 15% of the total product, is thought to be the RNA replicase. However, such a peak has not been detected in the cell-free product obtained with bacteriophage ZIK/RNA.

It has been shown that bacteriophage RNA can bind coat-protein subunits to give a complex that can be translated in the cell-free system to give coat protein but no RNA replicase (Sugiyama & Nakada, 1968; Lodish, 1968). To determine if any of the minor proteins synthesized in the cell-free system primed with bacteriophage ZIK/1 RNA arose from the RNA replicase cistron, coat-protein–RNA complexes were translated in the cell-free system. Bacteriophage ZIK/1 RNA–coat protein complexes were prepared by incubating purified RNA with different molar ratios of coat protein in 0.1M-tris–HCl, pH 8.1, at 37°C for 5 min. Fig. 5 shows incorporation of [^3H]histidine (9010 mCi/mmol) and [^{14}C]phenylalanine (225 mCi/mmol; The Radiochemical Centre) measured in the presence of complexes prepared with increasing quantities of coat protein. Incorporation of histidine shows a greater decrease than that of phenylalanine, in agreement with a selective inhibition of synthesis of non-coat protein. Fig. 6 shows the electrophoretic profiles of [^{14}C]phenylalanine-labelled proteins produced in the cell-free system in the presence of bacteriophage ZIK/1 RNA and a bacteriophage ZIK/1 RNA–coat protein complex. The minor protein peaks (fractions 1–21 in Fig. 6a)

Fig. 5. Effect of coat protein on the incorporation of [^{14}C]phenylalanine (○) and [^3H]histidine (●) in the *E. coli* cell-free system in the presence of bacteriophage ZIK/1 RNA

Coat protein–RNA complexes were prepared as described in the text and incorporation was measured in 0.05 ml reaction mixtures after incubation for 1 h at 37°C as described in the Methods section.

are greatly diminished or absent when the system is primed with the complex and it is therefore evident that these minor proteins arise from the replicase cistron. They may represent incomplete replicase molecules or degradation products; very little intact replicase is detected.

Discussion

The amino acid composition of bacteriophage ZIK/1 coat protein differs from those of other RNA bacteriophage proteins, although they have a common feature in the absence of histidine. It is not known whether this feature has any significance, but it could possibly be involved in control of bacteriophage-specific protein synthesis *in vivo*. Bacteriophages ZIK/1 and Q β belong to the same class on the basis of G+C content of RNA, but can be placed in different classes with regard to their coat proteins. Thus Q β -type bacteriophages lack histidine and methionine in the coat protein and ZIK/1-type bacteriophages lack histidine, methionine and cysteine. The bacteriophages in the higher (G+C) group are almost identical; bacteriophage f2 and R17 coat proteins differ in sequence in one amino acid out of 129 residues (f2 has methionine and R17 has leucine at position 88) (Weber & Konigsberg, 1967; Weber, 1967). It appears therefore that the RNA bacteriophages can be divided into three classes.

Fig. 6. Polyacrylamide-gel electrophoresis of proteins synthesized in the *E. coli* cell-free system in the presence of a bacteriophage ZIK/1 RNA-coat protein complex

(a) [^{14}C]Phenylalanine-labelled proteins produced in the presence of bacteriophage ZIK/1 RNA mixed with [^3H]valine-labelled purified coat protein (----); (b) [^{14}C]phenylalanine-labelled proteins produced in the presence of a coat protein-RNA complex. Bacteriophage ZIK/1 RNA (600 $\mu\text{g}/\text{ml}$) was mixed with purified coat protein in the ratio 1 mol of RNA: 25 mol of coat protein and incubated in 0.02 M-tris-HCl, pH 8.1, at 37°C for 5 min. Proteins were isolated and subjected to electrophoresis as described in the Methods section.

Analysis of the proteins produced in the *E. coli* cell-free system primed with bacteriophage ZIK/1 RNA gives a similar result to that obtained with RNA from bacteriophages f2 or MS2 (Nathans *et al.*, 1966; Eggen *et al.*, 1967; Vinuela *et al.*, 1967b) in that coat protein is the major product in each case. The bacteriophage ZIK/1 replicase cistron appears to yield several protein fragments but very little intact product. Lodish & Robertson (1969) have shown that in the cell-free system the bacteriophage f2 RNA replicase cistron yields three proteins, one of which is the intact replicase (about 50% of the cistron product). The failure to detect a substantial amount of intact replicase in the bacteriophage ZIK/1 product may mean that the bacteriophage ZIK/1 replicase is unstable or that few ribosomes manage to translate the whole cistron, possibly

because of the secondary structure of bacteriophage ZIK/1 RNA. The secondary structure of bacteriophage ZIK/1 RNA differs from that of bacteriophage MS2 RNA (Bishop, 1965) and modification of RNA secondary structure has a marked effect on replicase synthesis (Lodish, 1970).

There appears to be little difference between bacteriophage ZIK/1 RNA and f2-type RNA in the cell-free system, and it is therefore probable that the bacteriophage ZIK/1 genome is organized similarly to that of the f2-type bacteriophages. Coat proteins from bacteriophages R17 and Q β show some homology; allowing for deletions and insertions in the coat cistron 30 out of 131 residues in the protein can be made to correspond and 62 can be accounted for by a single base change (Konigsberg *et al.*, 1970). The 5'-terminal regions of RNA from bacteriophages R17 and Q β show a considerable degree of homology (Adams & Cory, 1970). The genomes of RNA bacteriophages appear to be structurally similar and to have the same functional organization. The differences between the types probably arise from minor differences in RNA sequence.

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