Acta virol. **28**: 257–266, 1984

POLYACRYLAMIDE GEL ELECTROPHORESIS OF THE KEMEROVO VIRUS RNA

I. SLÁVIK, I. BAČÍK, M. ROSENBERGOVÁ

Institute of Virology, Slovak Academy of Sciences, 817 03
Bratislava, Czechoslovakia

Received January 16, 1984

Summary. - Ribonuclease (RNase)-resistant RNA was isolated from partially purified Kemerovo virus by gel chromatography and or sucrose density gradient centrifugation. Double-stranded (ds) RNA only was found in the purified viral cores. The RNAs from both sources exhibited the same pattern of distribution in polyacrylamide gels. Ten dsRNA segments were identified. According to the results of coelectrophoresis of the Kemerovo virus and reovirus dsRNAs, the size of Kemerovo virus genome was estimated to be of about 11.7×10^6. The grouping of Kemerovo virus double-stranded segments according to their size in polyacrylamide gels corresponded to the 2:4:3:1 pattern.

Key words: Kemerovo virus; Dearing strain of reovirus type-3; double-stranded RNA; polyacrylamide gel electrophoresis

Introduction

The viruses serologically classified in Kemerovo subgroup of *Orbivirus* genus (Verwoerd *et al.*, 1979) were first isolated on the Eurasian, and later, on other continents. The possible dsRNA nature of Kemerovo virus (KV) genome material was evidenced by the finding of RNase-resistant form of RNA in partially purified viral preparations (Rosenbergová and Slávik, 1975).

In this paper we report on the further characterization of this RNA in polyacrylamide gels and on the presence of the same dsRNA in core particles derived from Kemerovo virions.

Materials and Methods

Chemicals. The compounds of following provenience and purity were used: Actinomycin D, Trizma base (Tris) — from Sigma; Triton X-100 (TX-100), puriss. — from Koch-Light; Sucrose, cryst., ultra pure, Sodium deoxycholate (SDC) — from Schwarz/Mann; Bentonite — from B.D.H.; Sephadex G-100, fine, Blue Dextran 2000 — from Pharmacia; Cetyltrimethylammonium bromide (CTAB) — from Merck; Acrylamide, 2xcryst., N, N'-Methylene bisacrylamide, 2xcryst., N, N, N', N'-Tetramethyl ethylenediamine (TMED), Ammonium persulphate, Sodium dodecylsulphate (SDS), Serva Blue G, Freon-113 — from Serva; Fraction V of bovine serum albumin (BSA), Ribonuclease, 5xcryst., A grade, Deoxyribonuclease (DNase) — from Calbiochem; Chymotrypsin — from Boehringer.

Viruses and cell cultures. The R-10 strain of KV of the same history as previously reported (Rosenbergová and Slávik, 1975) was used after 4–5 passages in brains of suckling rats. Brain suspensions were lyophilized after clarification at low-speed and stored at −18 °C.

Primary chick embryo cells (CEC) were cultivated on 10–40 Petri dishes of 150 mm internal diameter (Slávik *et al.*, 1983). Stock virus was resuspended in 7 mmol/l Tris-HCl-buffered saline (TBS) pH 7.6 (10 ml per dish) and clarified for 7 min at 1,500 g. The cells were infected for 90 min at ambient temperature with multiplicity of infection (m.o.i.) of about 0.1. Fresh serum-free basal Eagle's medium (BEM) buffered with 7 mmol/l Tris-HCl pH 7.7 at 24 °C was used to replace viral inoculum; then the cells were incubated for 24 hr at 37 °C. Rounded cells were scraped off into the nutrient fluid and pooled.

Approximately 8×10^6 BHK-21 cells were seeded in 20 ml of bicarbonate-buffered BEM containing 10% calf serum into a roller bottle of 180 cm^2 surface area. The number of roller bottles used per experiment ranged from 8–12. When the monolayers became confluent, the cells were infected with the stock KV resuspended in serum-free BEM for 90 min at m.o.i. of about 0.2. The cells were incubated for 18–20 hr at 37 °C in 15 ml of serum-free medium per bottle, and after cooling to 4 °C they were harvested by shaking off to the nutrient.

Reovirus type-3, strain Dearing, was maintained by serial passaging in BHK-21 cells. After sedimentation of infected cells the supernatant contained about 5% of infectivity. It was either stored at −18 °C until use, or it was applied immediately to noninfected cells (m.o.i. of about 0.5). After 90 min absorption at 37 °C, the inoculum was replaced with 15 ml of fresh BEM containing 2% calf serum; after incubation at 37 °C the cells were harvested at the appearance of cytopathic effect.

Radiosotopic labelling and assay of radioactivity. Labelling of KV with either ^3H-uridine in CEC (specific activity = 65 MBq/μmol, concentration = 65 kBq/ml) or with ^{32}P-carrier-free sodium orthophosphate in BHK-21 cells (concentration = 98 kBq/ml of BEM lacking inorganic phosphate) was performed in the presence of 0.5 μg/ml of actimomycin D in the nutrient (Shatkin and Rada, 1967). Medium containing the radiochemicals was administered to cells immediately after the removal of viral inoculum; labelling proceeded for 18–24 hr at 37 °C. The labelled compounds were supplied by the Institute for Research, Production and Application of Radiosisotopes, Prague.

Aliquots of tritium-containing liquid samples were precipitated with trichloroacetic acid (TCA) and counted on glass fibre paper discs in toluene-based scintillator (Slávik *et al.*, 1976). Polyacrylamide gel slices were extracted according to Helleiner and Wunner (1971) and dried at room temperature. The Cerenkow's radiation of ^{32}P was assayed according to Haviland and Bieber (1970). Scintillation counting was performed on Packard TriCarb, model 3390, scintillation spectometer.

Purification of KV by sucrose-cushion sedimentation. KV labelled with ^3H-uridine in CEC was purified by repeated sedimentation onto the 65% (w/v) sucrose cushion (Rosenbergová and Slávik, 1975). From the material banding on the top of sucrose, the tritium-labelled RNA of the KV was extracted. The same purification procedure was used for preparation of nonlabelled RNase-resistant RNA of KV (see below).

Purification of ^{32}P-labelled KV by differential centrifugation. Infected BHK-21 cells were sedimented for 60 min at 3,000 g. Cellular pellets were resuspended in 40 ml of 2 mmol/l Tris-HCl buffer (TCB) pH 8.6, homogenized in Glass Tissue Grinder (Bellco Glass Inc.) and centrifuged for 10 min at 800 g. Resulting pellets were resuspended in the same vol as above, homogenized, stored for 24 hr at 4 °C, and spun again for 10 min at 800 g. Virus-containing supernatants from both low-speed centrifugations were mixed and treated for 15 hr at 4 C with 5 μg/ml RNase and 12 μg/ml of DNase. The virus was then sedimented in rotor SW-25.1 of L-5 ultracentifuge (Beckman) for 2 hr at 23,000 rev/min through 2 ml of 5% (w/v) sucrose cushion. At the end of run, the supernatants were removed, tube-walls were washed with TBS, then with TCB, and finally, the sucrose cushions were aspirated. From the material in pellets, the ^{32}P-labelled RNA of KV was extracted.

Preparation of KV core particles. KV cores were prepared from the virus purified by modified Verwoerd's procedure (Verwoerd, 1969, Verwoerd *et al.*, 1972). Infected CEC were sedimented for 15 min at 35,000 g and pellets were resuspended in 1/10 of the original volume with TCB pH 8.8. After re-pelletation as above, the resulting pellets were suspended in 1/20 of the original volume and homogenized. The suspension was treated with 20 μg/ml of both RNase and DNase for 20 min at 27 °C, and sonicated twice for 1 min (Raytheon, Model DF-101). The suspension was then diluted with TCB to 1/5 of the original vol and centrifudged for 15 min at 35,000 g. The resuspension, sonification and centrifugation procedures were repeated twice and the resulting three supernatants were pooled. The protein concentration in this viral suspension was estimated according to Bradford (1976) using BSA as protein standard. The virus was then treated with TX-100 at 4 °C. Detergent was added to viral suspension until its concentration reached the concentration of protein, and the virus was immediately pelleted through 5 ml of 47% (w/v) sucrose cushion. Centrifugation was performed in the rotor SW-27 for 150 min at 23,000 rev/min. Pellets were resuspended in TCB, sonicated twice for 1 min, and virus was re-pelleted in the rotor SW-25.1 for 120 min at 24,000 rev/min through a 5 ml sucrose cushion. The second pellets were resuspended in TCB by sonification and treated with Freon-113 according to Verwoerd *et al.*, (1972), except that extraction proceeded in the absence of Sephadex. Viral cores were prepared from Freon-treated KV by chymotrypsin treatment (VanDijk and Huismans, 1980). Particles were sedimented in the 3 × 6.5 ml swing-out rotor (SW-60) of MSE-65 superspeed ultracentrifuge for 60 min at 30,000 rev/min through 0.5 ml of 47% sucrose cushion. The RNA of KV cores was extracted from the pelleted material.

Purification of reovirus. Reovirus was purified from the three times freezed and thawed and subsequently SDC-treated BHK-21 cells (Bellamy *et al.*, 1967). It was found that sufficient purity of reovirus dsRNA could be achieved when virus, clarified after SDC treatment, was once pelleted and then rebanded in CsCl density gradients. Sedimentation of virus to the equilibrium in preformed density gradients buffered with 7 mmol/l Tris-HCl pH 8.8 was done in the rotor SW-50.1 for 4 hr at 36,000 rev/min. Fractions of about 1.38 g . cm^{-3}-density were pooled and the virus was washed until substantially free of caesium ions on XM-300 membrane, of Amicon Model 12, Stirred Cell.

Extraction of RNA. KV purified by the above-described procedures and the CsCl-purified reovirus were suspended prior to RNA extraction in TBS pH 8.4 containing 2 mmol/l EDTA (TNE) and 1% of SDS. Phenol extraction of RNA was performed as previously described (Rosenbergová and Slávik, 1975). The RNAs were twice precipitated with 2.5 vol of ethanol for 18 hr at −18 °C prior to use. Core particles of KV were suspended in a solution containing 0.1 mol/l LiCl, 10 mmol/l Tris-HCl pH 8.4, 2 mmol/l EDTA and 1% lithium dodecylsulphate. RNA was extracted with phenol and it was once precipitated with ethanol prior to its analysis on polyacrylamide gels.

Isolation of RNase-resistant RNA of KV by gel chromatography. RNAs extracted from KV which had been purified either from CEC by sucrose cushion procedure or from BHK-21 cells by differential centrifugation, were separately fractionated on Sephadex G-100 column of 27 × 180 mm dimensions. Elution was done with TNE containing 0.01% of SDS and fractions of about 1.9 ml volume were collected. Chromatography was monitored at 254 nm (UV-Analyzer, Developmental Workshops of Czechoslovak Acad. Sci., Prague) and the fraction-collector was equipped by a drop counter (Type DC 1002, Laboratory Apparatuses, Prague). The aliquots of fractions were supplemeented with 0.2 mg of BSA prior to and after the RNase treatment (Rosenbergová and Slávik, 1975), and were precipitated into 1 ml with 10% TCA. The precipitates were filtered onto the glass fibre paper discs, washed with 5% TCA, then with ethanol and ethanol-ether and counted. The fractions containing RNase-resistant RNA were pooled and RNA was recovered by precipitation with CTAB (Ralph and Bellamy, 1964).

Sucrose density gradient centrifugation of the RNase-resistant KV RNA. The labelled RNase-resistant RNAs were separately analysed in 30 ml sucrose density gradients. Samples of 1 ml vol were layered over 29 ml of 15−30% (w/v) sucrose density gradients made in TNE containing 1 mg/ml of bentonite. Centrifugation proceeded in the rotor SW-25.1 for 25 hr at 24,000 rev/min and 4 °C. By the end of run, the tube content was fractionated from the bottom and monitored at 254 nm. The peaks of the marker ribosomal RNA (rRNA) from Ehrlich ascites tumour cells were detected photometrically and the viral RNAs were identified according to radioactivity. The viral RNAs were recovered by precipitation with CTAB.

Polyacrylamide gel electrophoresis (PAGE) procedures. Polyacrylamide gels were prepared from stock solution of recrystallized monomers (28.5 g, and 1.5 g per 100 ml, respectively), from vacuum-distilled TMED and from commercial ammonium persulphate. The 3% cylindrical or slab gels polymerized in Tris-acetate buffer (pH 7.5 at 20 °C) were prerun with the buffer containing 2 mmol/l EDTA (Loening, 1967). Electrophoreses run at about 20−22 °C in aparatuses equipped with cooling and through-flow of reservoir buffer. The cylindrical 6 × 80 mm gels (Slávik *et al.*, 1976) were loaded with samples containing glycerol and bromophenol blue and electrophoresed for 4.5 hr at 10 V/cm. At the end of run, the gels were stained with methylene blue (Peacock and Dingman, 1967), photographed, and freeze-sectioned to 1 mm thick slices. They were placed onto glass fibre paper discs and extracted as described above prior to counting.

Coelectrophoresis of RNase-resistant KV and reovirus RNAs was done on polyacrylamide slab gels 150 × 110 × 2 mm in size (width × height × depth) at 8 V/cm. At the end of run, the gels were stained with Pyronin Y and photographed.

The separation of KV dsRNA segments of similar molecular masses was performed on 40 cm-long gels buffered according to Laemmli (1970). The 3% gels of 0.4 mm thickness were shortly prerun with buffer containing 2 mmol/l EDTA. The samples of about 10 μl were applied and electrophoresed in noncooled apparatus for 7–20 hr at 100–300 V and about 5–20 mA. At the end of run, the gels were stained with 0.1% toluidin blue 0, washed, sticked onto Whatman 3MM paper and photographed. The 5% gels containing RNA of KV cores and reovirus RNA in the same lane were covered after staining with Saran Wrap. The distances corresponding to respective dsRNA segments were directly measured on wet gel; the aim of this was to eliminate small shifts probably introduced by photographic procedures.

The values of M_r 2.5, 2.4, 2.3, 1.6, 1.6, 1.4, 0.92, 0.76, 0.64 and 0.61×10^6, reported for dsRNA segments of reovirus, type 3 (strain Dearing, Ramig *et al.*, 1977) were used for the construction of the standard curve to estimate the apparent molecular masses of KV dsRNA segments.

Results

Gel chromatography of KV RNA

Radioactively labelled RNA of KV purified from CEC by the sucrose cushion procedure yielded several peaks upon gel chromatography on Sephadex column. The RNase-resistant form of KV RNA, which was further analysed in sucrose density gradients and in PAGE, eluted immediately behind the void volume-peak of Blue Dextran 2000 (Fig. 1). The RNase-sensitive species of RNA eluted with the main peak between fractions 28 and 32, i.e. in an elution volume of about 60 ml. Smaller peaks of RNase-sensitive RNA also eluted near to the peak-fractions No. 25 and 34, i.e. in elution volumes of about 45 ml, and 74 ml, respectively (Fig. 1).

Sucrose density gradient sedimentation of RNase-resistant KV RNA

The RNase-resistant KV RNA sedimented in 30 ml sucrose density gradient in a broad zone with the main peak at 12 S (Fig. 2). On both sides of the main peak, shoulders of radioactivity appeared at about 14 S, and 10.5 S, and small peaks of radioactivity at about 16 S, and 9.5 S, respectively.

The sedimentation properties were evaluated for dsRNA segments of KV core RNA according to the data previously published on dsRNAs of other viruses (see reference to Table 1). When the sedimentation constants were put in correlation with the radioactivity profile of RNase-resistant RNA in sucrose density gradient, good agreement was achieved between the theoretical data and the experimental findings (Fig. 2, insert).

Polyacrylamide gel electrophoresis of the RNase-resistant KV RNA

The labelled RNase-resistant KV RNAs were electrophoresed in cylindrical polyacrylamide gels. Both, the tritium-labelled RNA isolated by gel chromatography, and the ^{32}P-labelled RNA purified on Sephadex column and also in the sucrose density gradient, exhibited nine bands upon staining and corresponding radioactivity in cylindrical polyacrylamide gels (Fig. 3, I and II). The radioactivity in the fourth peak indicated the presence of 2—3 bands corresponding to two bands demonstrated by staining.

In the gel containing ^{32}P-RNA, also non-radioactive rRNAs from the sucrose density gradient fractions were visualized (Fig. 3—II, insert).

Coelectrophoresis of RNase-resistant KV and reovirus RNAs

Electrophoresis of KV and reovirus RNAs in the same track of the polyacrylamide slab gel was used for evaluation of apparent molecular masses of RNase-resistant RNA species of KV (Figs 4-I and -II). The apparent M_r values of about 2.7, 2.0, 1.35, 1.15—1.11, 0.7, 0.65, 0.57, and 0.25×10^6 were found. The M_r values for the RNase-resistant KV RNA were practically

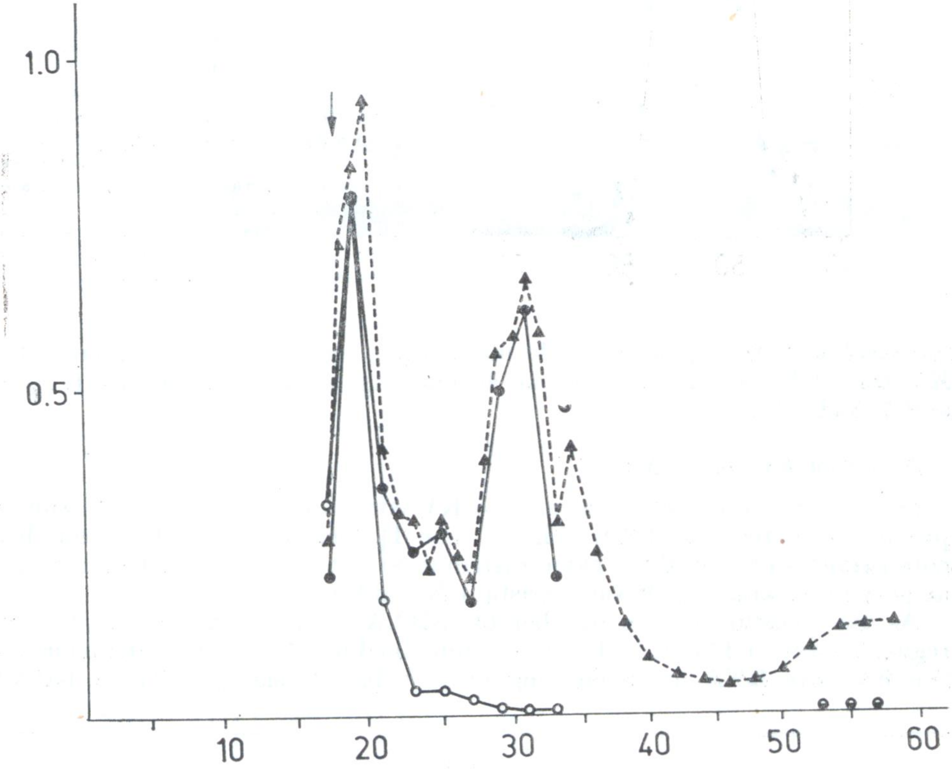

Fig. 1.
Gel chromatography of ^{3}H-labelled KV RNA on Sephadex G-100 colun.
Acid-precipitable radioactivity of the fractions prior to (●——●) and after (○——○) RNase treatment; radioactivity of the fraction-aliquots not precipitated with TCA (▲— — —▲).
Arrow represents the peak of Blue Dextran 2000.
Abscissa: fraction number; ordinate: c.p.m. × 10^{-3}.

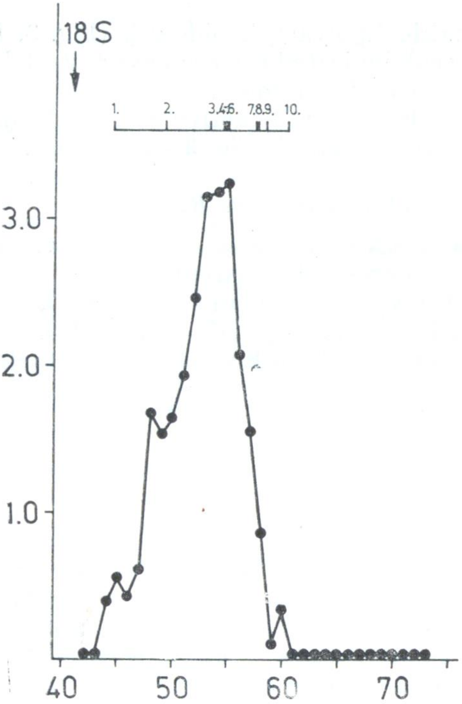

Fig. 2.
Sedimentation of RNase-resistant KV RNA (fractions No. 17—21 from Fig. 1) in a 30 ml sucrose density gradient
Arrow represents the 18 S rRNA peak.
Abscissa: fraction number; ordinate: c.p.m. × 10^{-2}.
Insert: numbers indicate the appropriate dsRNAs with molecular masses and sedimentation constants given in Table 1.

identical with the apparent molecular masses more precisely estimated for KV core dsRNA segments on the 40 cm-long polyacrylamide gels (Figs 6 and 7, Table 1).

PAGE of KV core RNA

In the preliminary experiment, the KV core RNA (Fig. 5, lane 2) run in parallel with reovirus RNA (Fig. 5, lane 1). The RNA isolated from KV core particles exhibited the same distribution pattern in polyacrylamide gel as previously analysed RNase-resistant KV RNA.

As the question of the number of dsRNA segments present in the M_r region between 1.15 and 1.11 × 10^6 has remained unsolved, we electrophoresed the KV core RNA in 40 cm-long polyacrylamide slab gel. Three dsRNA

Fig. 3
Electrophoresis of radioactively labelled RNase-resistant KV RNA on cylindrical polyacrylamide gels
Above (I) — ^{3}H-KV RNA; below II — ^{32}P-KV RNA.
Abscissa: fraction number; ordinates: c.p.m. × 10^{-3}.
Photographic inserts above and below show the distribution on the ^{3}H- and ^{32}P-KV RNA according to staining.
In insert below also the RNAs from sucrose density gradient fractions are visualized.

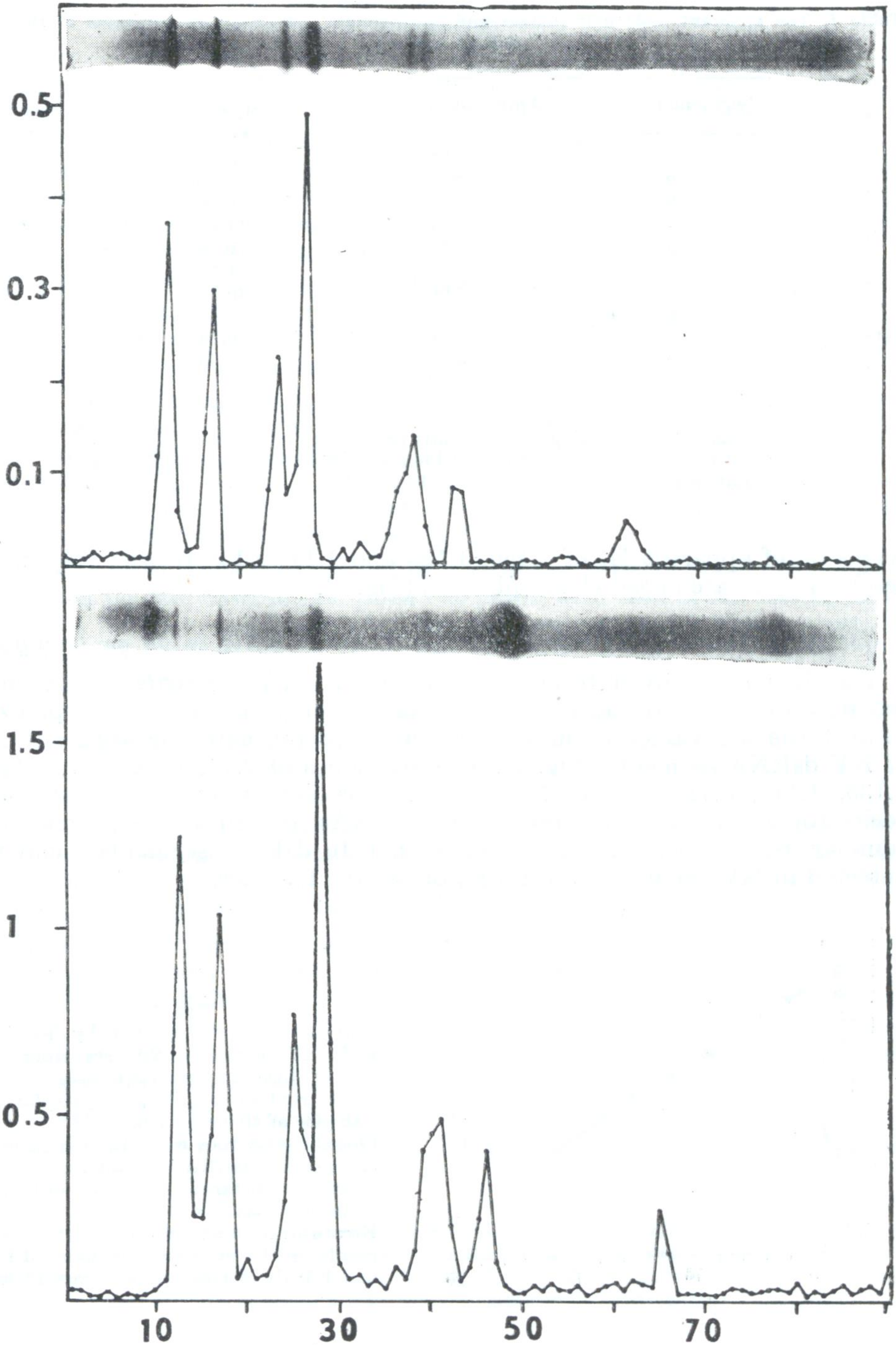
0.5
0.3
0.1
1.5
1
0.5
10
30
50
70

Table 1. The apparent molecular masses and approximate sedimentation constants of the KV dsRNA segments

Segment number	Apparent $M_r \times 10^6$	S value
1	2.71*	16.30**
2	1.95	14.14
3	1.37	12.48
4	1.150	11.82
5	1.135	11.78
6	1.111	11.72
7	0.718	10.58
8	0.678	10.47
9	0.568	10.18
10	0.270	9.30
Sum	11.66	

*Calculated according to the results presented in Fig. 7.

**According to data of Katz and Penman (1966), Franklin (1967) and Bellamy *et al.* (1967).

segments of apparent M_r of 1.150, 1.135 and 1.111×10^6, respectively, were resolved upon prolonged electrophoresis (Fig. 6).

Coelectrophoresis of KV core RNA and reovirus RNA in 40 cm-long gels. The distances moved from the start by dsRNA segments of KV and reovirus were directly measured on wet polyacrylamide gel and plotted against the log values of molecular masses to estimate the apparent M_r of KV dsRNA segments (Fig. 7). The M_r values of 2.71, 1.95, 1.37, 1.150, 1.135, 1.111, 0.71, 0.68, 0.57, and 0.27×10^6 were found. The results of coelectrophoresis of KV and reovirus RNAs on 40 cm-long gels are summarized in Table 1. We conclude that 10 dsRNA segments could be detected in KV genome of a total M_r of about 11.7×10^6.

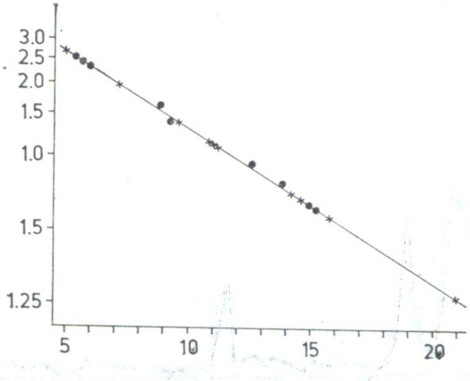

Fig. 7.

Plot of distances moved by reovirus and KV core dsRNA segments vs. logs of molecular masses

Coelectrophoresis in 5% polyacrylamide slab gels of 40 cm length.

Closed circles: reovirus dsRNA segments; crosses: KV dsRNA segments.

Abscissa: distances in cm; ordinate: molecular masses $\times 10^{-6}$.

Erratum: by mistake the first two segments on the ordinate were marked 1.25 and 1.5; the correct values are 0.25 and 0.5

Discussion

The procedures used for partial purification of KV, i.e. the sucrose-cushion and the differential centrifugation procedures, yielded viral samples containing ssRNA (Rosenbergová and Slávik, 1975; Fig. 1 in this communication). As in the presence of actinomycin D, most of newly synthesized RNA in reovirus-infected cells was of viral provenience (Shatkin and Rada, 1967), it seems that the RNase-sensitive KV RNA (Fig. 1) may correspond mainly to in vivo-produced KV transcripts.

The KV ^{32}P-RNA purified from BHK cells by differential centrifugation contained only small amount of ssRNA when chromatographed on Sephadex column (results not shown). The peak appearing at the total volume of the column indicated that some portion of KV RNA disintegrated probably due to the decay of the incorporated radioactive phosphorus. In sucrose density gradient a peak at about 6 S appereared probably corresponding again to degraded RNase-resistant KV RNA (not shown). The ^{32}P-RNase-resistant KV RNA, which was further analysed in cylindrical polyacrylamide gel (Fig. 3-II) was, therefore, isolated from the sucrose fractions present in the sedimentation range between 8.5 and 16 S.

The finding of segmented dsRNA in KV cores and its identical distribution pattern in polyacrylamide gels when compared with that of RNase-resistant KV RNA led us to conclusion that the previously reported RNase-resistant RNA of KV (Rosenbergová and Slávik, 1975) actually represented the genomic KV RNA.

In the size of the dsRNA genome and in the number of the ten dsRNA segments, the KV did not differ from other Orbiviruses studied so far (Verwoerd *et al.*, 1979). However, when considering the molecular masses of KV dsRNA segments, and thereby, their grouping in polyacrylamide gels, it appears that the genomic dsRNA of KV consists of two large, four medium, three small and one very small segments, respectively. The size distribution of genomic dsRNAs of orbiviruses in general corresponds rather to the 3 : 3 :3:1 pattern (Verwoerd *et al.*, 1979).

The coding capacity of dsRNA viral genomes is usually expressed by the RNA-to-protein mass ratio of 18 : 1. This figure seems to be generally accepted. Its validity was exemplified also with bluetongue virus, which dsRNA (M_r of about 12×10^6) codes for proteins of total M_r of 6.66×10^5 (Huismans, 1979). When applied for the KV model, the 11.7×10^6 genome could code for proteins with total molecular mass of about 6.5×10^5.

The existence of four medium dsRNA segments in KV genome might result in the presence of one structural polypeptide of lower M_r in the Kemerovo virion than in other orbiviruses.

Acknowledgment. We would like to express our thanks to Dr. H. Libíková and Ing. G. Ruttkay-Nedecký for their interest in this study and for their help in the preparation of manuscript. We are indebted to Miss I. Zikmundová an Mrs. M. Dostálová for the technical assistance, and to Mr. P. Kvíčala and Mr. N. Dokoupil for the photographic documentation.

References

Bellamy, A. R., Shapiro, L., August, J. T., and Joklik, W. K. (1967): Studies on reovirus RNA. I. Characterization of reovirus genome RNA. *J. mol. Biol.* **29**, 1—17.

Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72**, 248—254.

Franklin, R. M. (1967): Replication of bacteriophage ribonucleic acid: Some physical properties of single-stranded, double-stranded and branched viral ribonucleic acid. *J. Virol.* **1**, 64—75.

Haviland, R. T., and Bieber, L. L. (1970): Scintillation counting of ^{32}P without added scintillator in aqueous solutions and organic solvents and on dry chromatographic media. *Analyt. Biochem.* **33**, 323—334.

Helleiner, C. W., and Wunner, W. H. (1971): A simple method for counting ^{14}C- and ^{3}H-proteins in polyacrylamide gels. *Analyt. Biochem.* **39**, 333—338.

Huismans, H. (1979): Protein synthesis in bluetongue virus-infected cells. *Virology* **92**, 385—396.

Katz, L., and Penman, S. (1966): The solvent denaturation of double-stranded RNA from poliovirus infected HeLa cells. *Biochem. biophys. Res. Commun.* **23**, 557—560.

Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* **227**, 680—695.

Loening, U. E. (1967): The fractionation of high-molecular-weight ribonucleic acid by polyacrylamide-gel electrophoresis. *Biochem. J.* **102**, 251—257.

Peacock, A. D., and Dingman, C. W. (1967): Resolution of multiple ribonucleic le acid species by polyacrylamide gel electrophoresis. *Biochemistry* **6**, 1818—1827.

Ralph, R. K., and Bellamy, A. R. (1964): Isolation and purification of undegraded ribonucleic acid. *Biochem. biophys. Acta* **87**, 9—16.

Ramig, R. F., Cross, R. K., and Fields, B. N. (1977): Genome RNAs and polypeptides of reovirus serotypes 1, 2 and 3. *J. Virol.* **22**, 726—733.

Rosenbergová, M., and Slávik, I. (1975): Ribonuclease-resistant RNA of Kemerovo virus. *Acta virol.* **19**, 67—72.

Shatkin, A. J., and Rada, B. (1967): Reovirus-directed ribonucleic acid synthesis in infected L cells. *J. Virol.* **1**, 24—35.

Slávik, I., Krasnobayeva, Z. N., Gaidamovich, S. Ya., and Melnikova, E. E. (1976): Composition of Zaysan virion. *Acta virol.* **20**, 177—182.

Slávik, I., Čiampor, F., and Mayer, V. (1983): Optimalized production of tick-borne enecephalitis virus in vitro. *Acta virol.* **27**, 97—104.

Van Dijk, A. A., and Huismans, H. (1980): The in vitro activation and further characterization of the bluetongue virus-associated transcriptase. *Virology* **104**, 347—356.

Verwoerd, D. W. (1969): Purification and characterization of bluetongue virus. *Virology* **38**, 203—212.

Verwoerd, D. W., Els, H. J., deVilliers, E. -M., and Huismans, H. (1972): Structure of the bluetongue virus capsid. *J. Virol.* **10**, 783—794.

Verwoerd, D. W., Huismans, H., and Erasmus, B. J. (1979): Orbiviruses, pp. 285—345. In H. Fraenkel-Conrat and R. R. Wagner (Eds): *Comprehensive Virology* 14. Plenum Press, New York, London.

Explanation of Figures (Plates XLII—XLIII):

Fig. 4. Coelectrophoresis of RNase-resistant KV and reovirus RNAs in polyacrylamide slab gels stained with Pyronin Y.

I. Lane 1: KV RNA; lane 2: mixture of KV and reovirus RNAs; lane 3: reovirus RNA.

II. Mixture of KV and reovirus RNA electrophoresed to a 1.5 times longer distance than in I; dsRNA segments of KV and reovirus are drawn on the same sides of lane 2 as in 4-I.

Fig. 5. Polyacrylamide slab gel electrophoresis of KV core RNA and reovirus RNA on 3% gel stained with Pyronin Y. Lane 1: reovirus RNA; lane 2: KV core RNA.

Fig. 6. PAGE of KV core RNA in 40 cm long 3% gels stained with toluidine blue 0.
Lane 1: about 40 μg of RNA; lane 2: about 20 μg of RNA.