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ELECTROPHORESIS OF THE COMPLEMENTARY STRANDS OF THE DOUBLE-STRANDED KEMEROVO VIRUS RNAs IN AGAROSE-UREA GEL

I. BAČÍK

Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Czechoslovakia

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Summary. — Single-stranded (ss)RNAs derived from 10 double-stranded (ds)RNA segments of Kemerovo virus (KV) were separated into 13 RNA bands by agarose-urea gel electrophoresis. The complementary strands of the dsRNA segments 1,9 and 10 displayed different electrophoretic mobility. An attempt was made to determine the origin of the ssRNA bands. The ssRNA bands originating from the dsRNA segments 1, 2, 3, 9 and 10 were identified unequivocally, while those originating from the dsRNA segments 4, 5, 6, 7 and 8 were characterized partially. The minus RNA strands of the dsRNA segments 9 and 10 exhibited higher electrophoretic mobilities as their complementary plus RNA strands.

Key words: *Kemerovo virus*; *agarose-urea gel electrophoresis*; *double-stranded RNA segments*; *complementary RNA strands*

Introduction

Viruses of the Kemerovo serogroup, members of the family *Reoviridae* (Verwoerd *et al.*, 1979), contain 10 genomic dsRNA segments (Gorman *et al.*, 1983; Slávik *et al.*, 1984). The separation of the plus and minus strands of the genomic dsRNA segments by agarose-urea gel electrophoresis (Smith *et al.*, 1981) showed that plus strands of cytoplasmic polyhedrosis virus (CPV) and most reovirus minus strands migrated faster than their complementary strands of opposite polarity. Previously, Patton and Stacy-Phipps (1986) reported higher electrophoretic mobilities of all rotavirus plus strands than their corresponding minus strand RNAs. The identity of the plus strands of genomic dsRNA segments with the mRNA was referred for viruses such as reovirus, bluetongue virus, CPV or rotavirus (Skehel and Joklik, 1969; Van Dijk and Huismans, 1980; Smith *et al.*, 1981; Imai *et al.*, 1983). Both, plus strands of dsRNA segments and also mRNAs, respectively, may possess cap structures at their 5′ends (Furuichi *et al.*, 1975; Furuichi and Miura, 1975; Imai *et al.*, 1983). Recently, we reported the possibilities of selective labelling of one (minus) RNA strand only, or both complementary RNA strands (plus and minus RNA strands) in KV dsRNA segments; a 5′-terminal

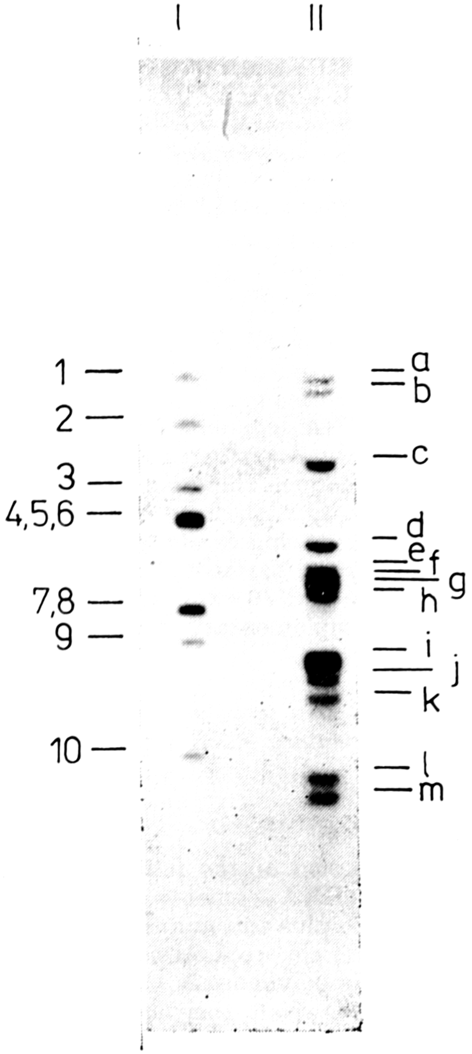

Fig. 1

Separation of KV genome RNAs by electrophoresis in 1.75% agarose gel containing 7 mol/l urea

dsRNA segments were ^{32}P-pCp-labelled at 3′ ends. Lane I: Native dsRNA segments of KV; lane II: ssRNA derived from dsRNA segments by heat-denaturation in 7 mol/l urea.

modification of KV genomic plus RNA strands was demonstrated by separation of complementary genomic RNA strands in agarose-urea gel electrophoresis (Bačík, 1990). In this work the electrophoretic analysis of the genomic complementary RNA strands is presented.

Materials and Methods

dsRNAs of KV (R-10 strain) were prepared as described (Bačík, 1990).

Labelling of the 3′ends of dsRNA segments by 5′-^{32}P-pCp (Amersham) using T4 RNA ligase (PL-Biochemicals) was performed as described by D'Alessio (1982).

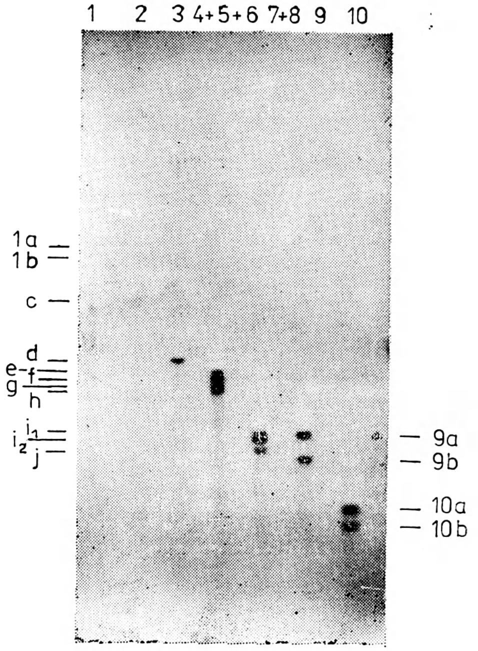

Fig. 2

Resolution of ssRNAs derived from KV dsRNA segments by electrophoresis in agarose-urea gel

^{32}pCp-labelled dsRNA bands were recovered from gel slices, heat denatured and electrophoresed. Lanes 1–10 correspond to dsRNA segments 1–10.

Dephosphorylation of the 5'ends of dsRNA segments was made by treatment with calf intestinal alkaline phosphatase (Sigma) (Donis-Keller *et al.*, 1977; Maniatis *et al.*, 1982; Bačík, 1990).

Labelling of the 5'ends of dsRNAs was made with gama-^{32}P-ATP (Amersham) using T4 polynucleotide kinase (New England Nuclear) as described by Maniatis *et al.* (1982).

Agarose-urea gel electrophoresis of RNA was performed according to Smith and Furuichi (1980), Smith *et al.* (1981) and Bačík (1990). Agarose and urea were obtained from Lachema (Czechoslovakia).

Autoradiography of ^{32}P-labelled RNAs was done with wet gels and Medix-Rapid X-ray film (Foma, Czechoslovakia), at 4°C.

Recovery of the dsRNA segments from the agarose-urea gel. RNA-containing slices of a volume of about 0.15 ml, were cut out from the gel and placed into tubes with 1.5–2.0 ml of 2.5 mol/l urea and 0.5 mol/l CH_3COONa. Equal volume of water-saturated phenol was added and gel slices were thawed at 60–65°C for 5 min. After vigorous mixing, the samples were cooled in ice-bath and phases were separated by centrifugation. Water phase was phenol-extracted once again at room temperature and the RNA was obtained by ethanol precipitation.

Results

Separation of KV genomic RNAs

Ten KV dsRNA segments (Slávik *et al.*, 1984), labelled at 3'ends with 5'-^{32}P-pCp, were electrophoretically separated on agarose-urea gel into 7 bands (Fig. 1-I). The dsRNA segments 4, 5, 6 and also 7, 8 could not been distinguished from each other by this procedure. Single-stranded RNAs obtained from ten dsRNA segments by heat denaturation in 7 mol/l urea,

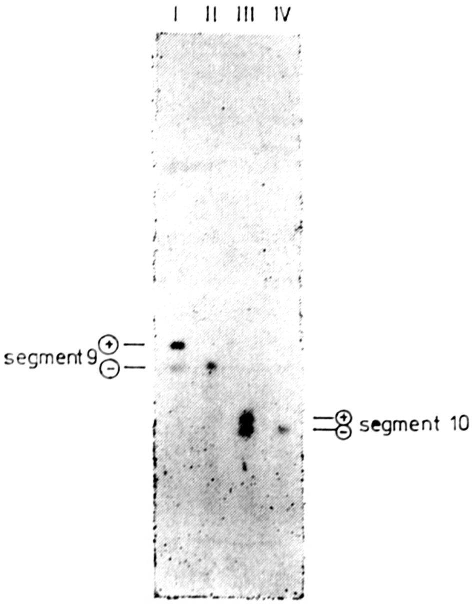

Fig. 3

Determination of the polarity of separated complementary strands of dsRNA segments 9 and 10

Both complementary RNA strands of dsRNA segments 9 (lane I) and 10 (lane III, were ^{32}P-labelled at 5′ ends, or only the unblocked (minus) RNA strands in dsRNA segments 9 (lane II) and 10 (lane IV) were ^{32}P-labelled at 5′ ends. + or − indicates the polarity of the RNA strand segment.

formed 13 bands — *a, b, c, d, e, f, g, h, i, j, k, l* and *m* (Fig. 1-II), thus the complementary RNA strands of certain dsRNA segments were separated.

Segmental origin of the RNA bands

In order to determine the segmental origin of 13 ssRNA bands (Fig. 1-II) slices containing dsRNA segments 1, 2, 3, 4+5+6, 7+8, 9 and 10 (Fig. 1-I) were cut out from the gel and the dsRNA was recovered by phenol-urea extraction and ethanol precipitation. Each dsRNA sample was heatdenatured in 7 mol/l urea and then electrophoresed. The complementary strands of dsRNA segments 1, 9 and 10 were separated into discrete RNA bands (Fig. 2). The complementary strands of the dsRNA segments 2 and 3 were not separated. The bands *e, f, g* and *h* contain ssRNAs derived from dsRNA segments 4+5+6. Each of the bands *f* and *g* contains probably two different ssRNA strands, judging according to their intensity as compared with bands *e* and *h*. The ssRNAs derived from dsRNA segments 7+8 were separated into bands i_1, i_2 and *j*. Band i_2 consisted probably of two different RNA strands. The electrophoretic mobility of bands i_1 and 9*a* (Fig. 2) was the same. This means, that band *i* (Fig. 1) contains also the ssRNA of dsRNA segment 9 (9*a*, Fig. 2). The bands *k* (Fig. 1) and 9*b* (Fig. 2) correspond each other.

Determination of the polarity of ssRNAs

The polarity determination of separated complementary RNA strands of dsRNA segment 9 and also 10 was achieved using dsRNA segments in which only one (minus) RNA strand was labelled at the 5′end or both RNA strands (plus and minus) were labelled at the 5′ends (Bačík, 1990). After heat denaturation of dsRNA segments in 7 mol/l urea and separation of complementary RNA strands in agarose-urea gels we observed that minus strands of these segments displayed higher electrophoretic mobility then corresponding plus strands (Fig. 3). This means, that bands *9a* and *10a* (Fig. 2) contain plus strands of dsRNA segments 9 and 10 respectively, and bands *9b* and *10b* contain the respective minus strands.

Discussion

Agarose-urea gel electrophoresis of RNAs, as described by Smith and Furuichi (1980) and Smith *et al.* (1981), seems to be useful for separation of complementary strands of KV dsRNA segments 1, 9 and 10. In the case of plus and minus strands of the segment 9 the difference in their electrophoretic mobility seems to be the largest. The electrophoretic mobility of some complementary strands of the segments 7 and 8 is higher than that of the plus strand of the segment 9 and lower than that of the minus strand of the segment 9. Complementary strands of dsRNA segments 2 and 3, and some ssRNA derived from segments 4, 5, 6 and also 7, 8 had the same electrophoretic mobilities. There is no evidence on the *in vitro* production of KV mRNAs as well as on their characterization from the *in vivo* systems. Using our experimental approach, the polarity of complementary RNA strands can be determined exploiting the presence of 5′terminal modification of plus RNA strands in dsRNA segments. The elaborated method for recovering of dsRNA segments from agarose-urea gels requires further improvement, namely an optimalization and quantification.

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