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Radish mosaic virus VPg

Characteristics and linkage with virion RNAs

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Comoviruses have a bipartite RNA genome. It is suggested that a small protein, VPg, is covalently linked to both RNAs. We have found that both radish mosaic virus RNAs are linked to identical VPg molecules via a phosphodiester bond between their 5'-terminal nucleotides and a serine residue of VPg.

Comovirus; Radish mosaic virus; VPg protein; RNA-protein complex; Phosphodiester bond

1. INTRODUCTION

Radish mosaic virus (RaMV), a member of the comovirus family, has a bipartite 'plus' single-stranded RNA genome. Both RNAs (B-RNA, ~2 MDa and M-RNA, ~1.2 MDa) are tightly linked to a small (~4 kDa) protein, denoted VPg [1,2].

It was found that in the strategy of the genome and the primary structure of non-structural proteins (a.o. VPg), the cowpea mosaic virus (CPMV), another member of the comoviruses, resembles picornaviruses [3,4]. Picornaviral VPg is bound to the RNA via a phosphodiester bond between its tyrosine residue and the 5'-terminal uridylate (review [5]). The complete amino acid sequence of CPMV VPg was deduced from its partial microsequencing and the primary structure of B-RNA. It was suggested that a tyrosine is not involved in the phosphodiester bond with CPMV RNA [6].

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Here, we found that RaMV VPg is linked with virion RNAs via a phosphodiester bond between a serine residue and the 5'-terminal nucleotide.

2. MATERIALS AND METHODS

^{32}P-labeled RaMV RNA was prepared according to the following protocol: 2 days after infection with RaMV a loba [1] leaf was cut off under water and placed into an Eppendorf tube with 0.5–1 mCi [^{32}P]orthophosphoric acid neutralized with KOH. After complete absorption of radioactivity, the leaf was transferred into a vial with phosphate-deficient Hoagland medium for 5–7 days incubation at 20°C. Virus and virion RNA isolation and complete hydrolysis of RNA with RNases or alkali were carried out as in [2].

RNA was electrophoresed in a 2% agarose denaturating gel at 4°C (75% DMSO, 25 mM LiCl, 40 mM Tris-acetate, pH 7.5, 1 mM EDTA [7]).

Incubation with RNase T_1 (2 U/μg RNA) was for 30 min at 37°C in 50 mM Tris-HCl, pH 7.5. The reaction was stopped by phenol deproteinization of RNA.

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Radiolabeled VPg-pNp from RNA hydrolysates or gel slices was extracted three times with water-saturated phenol/chloroform (1:1, v/v). It was transferred into the organic phase. VPg was precipitated from the phenol phase with 4 vols acetone in the presence of carrier BSA (10 μg/ml) and then dissolved in a minimal volume of 50 mM Tris-borate, 0.1% SDS. This solution was gel-filtered through Biogel P4 equilibrated with 0.1% SDS.

TPCK-trypsin digestion of ^{125}I-VPg-pNp was carried out in the presence of BSA (5 mg/ml) for 18 h at 37°C and at an enzyme concentration of 150 μg/ml in 100 mM NH_4HCO_3, pH 7.8.

3. RESULTS AND DISCUSSION

3.1. *Labeling VPg with Na^{125}I-chloramine T*

In a previous paper we showed that RaMV RNA-VPg can be labeled with ^{125}I-Bolton-Hunter reagent [2]. We also found that both B- and M-RNA preparations of RaMV were labeled by Na^{125}I-chloramine T under conditions commonly used for protein labeling (fig.1). The proteinase K treatment of labeled RNAs removed the ^{125}I label completely, leaving both RNAs intact. Therefore, RaMV VPg appears to contain aromatic amino acids and/or a histidine which can be labeled by Na^{125}I-chloramine T. After treatment with proteinase K (fig.1), the labeled amino acids are not directly linked to RaMV RNAs.

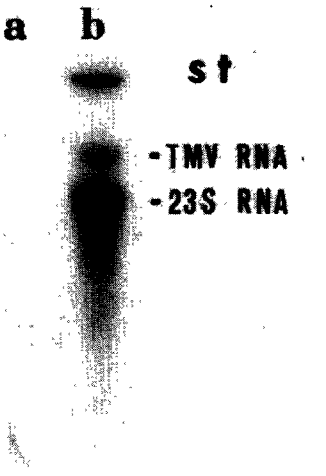

Fig.1. DMSO-LiCl gel electrophoresis of virion RaMV RNAs labeled with Na^{125}I-chloramine T. Lanes: a, RNA preparation treated with proteinase K (0.5 mg/ml, 37°C, 1 h); b, no proteinase K; st, start.

3.2. *B- and M-VPgs of RaMV are identical*

B- and M-RNA bands were cut off from a gel and incubated with RNases for 15–18 h at 37°C. The products of hydrolysis were analyzed by polyacrylamide gel electrophoresis. The complete hydrolysis of both RNAs under these conditions was proved by PAGE, so B- and M-VPgs-pNp were produced (see figs 3,4). TPCK-trypsin treatment of B- and M-^{125}I-VPg resulted in ^{125}I-peptides which were compared by two-dimensional mapping as in [8]. Both VPgs revealed no differences (fig.2). It was reported that the identical VPg molecules were bound to B- and M-RNA of cowpea mosaic virus [9]. Hence the conclusion that, in general, comoviruses have the same VPg molecules bound to each of their virion RNAs seems valid.

3.3. *Amino acid composition of VPg*

Six ^{125}I-peptides were found after the trypsin digestion of VPg by two-dimensional mapping. It follows from the amino acid analysis that RaMV VPg has a high content of proline (~10%), dicarboxylic amino acids and glycine with the result that the tryptic hydrolysis of VPg was limited and a higher number of peptides than one could expect from the tyrosine and histidine content (table 1) was produced. It is important that under the conditions of hydrolysis used a standard peptide pattern of lysozyme and TMV coat protein was observed. A comparison of RaMV and CPMV [10] genome-linked proteins (table 1) showed that RaMV VPg has a higher M_r and is slightly more hydrophobic and apparently less charged.

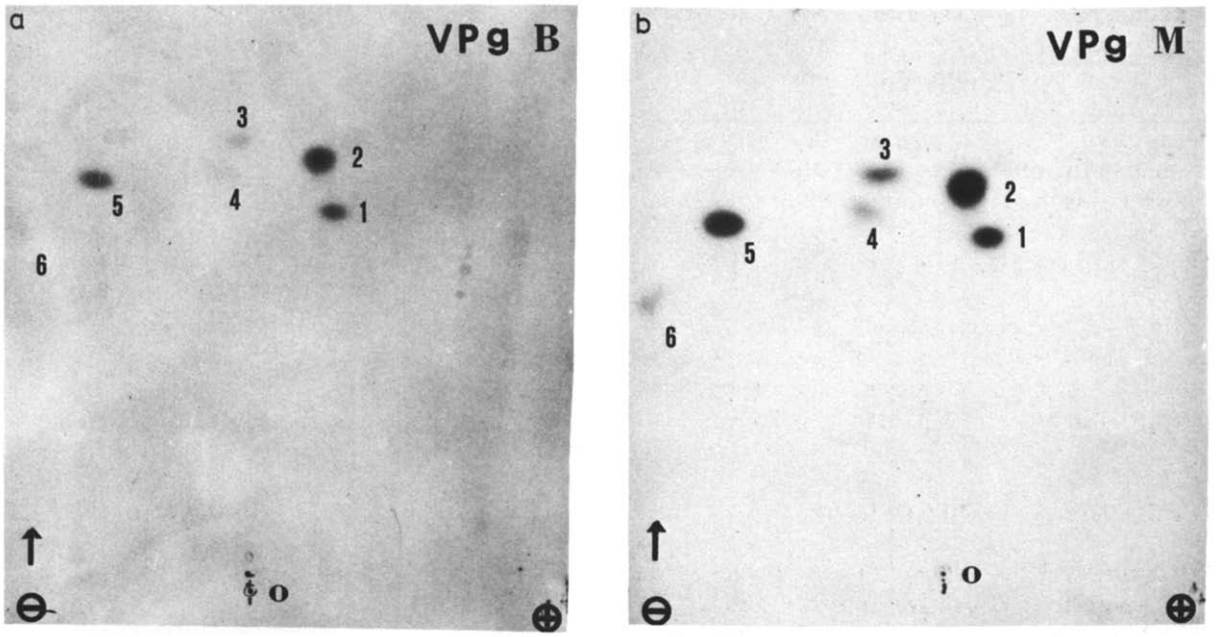

Fig.2. Tryptic maps of RaMV ^{125}I-VPg linked with: a, B-RNA; b, M-RNA. (○) Origin of sample application on a thin-layer cellulose plate.

A more detailed study of this difference is in progress.

3.4. *RaMV VPg is covalently linked with 5′-ends of RNAs via a serine residue*

The resistance of the RNA-VPg complex to SDS-phenol deproteinization is necessary but not sufficient proof of a covalent bond between RNA and VPg. More solid evidence can be obtained by isolating the unit of linkage and/or, at least, VPg linked specifically with a mono- or oligonucleotide. For this purpose two experiments were performed.

(i) ^{125}I-VPg-labeled B- and M-RNAs were treated with RNase T_1 or with a mixture of RNases, and the hydrolysis products were analyzed by urea-SDS-PAGE. As seen in fig.3 (lanes c,d), VPg remained bound to RNase T_1-derived oligonucleotide. Furthermore, the T_1-oligonucleotides produced from B- and M-RNA have the same length. It was reported earlier that CPMV 5′-terminal B- and M-T_1-oligonucleotides linked with VPg also had the same length [11].

(ii) RaMV virion RNAs were labeled in vivo with [^{32}P]orthophosphate. [^{32}P]RNA-VPg was treated with alkaline phosphatase (to remove putative monophosphate groups from VPg) before or after complete digestion of RNA with RNases. Again, the nucleotide ^{32}P label was linked with VPg under dissociating conditions (fig.4).

The final proof of the covalent linkage between RaMV VPg and RNAs was obtained in the following series of experiments. Genome [^{32}P]RNA-VPg was hydrolyzed completely by nuclease PI or by a mixture of RNases with subsequent phosphatase treatment. Both procedures produced the same result. Then, VPg linked with ^{32}P label was treated with snake venom phosphodiesterase (SVP). Most of the ^{32}P label was split off from VPg and found to be pU, pA and pG in the approximate ratio 5:4:1 (not shown). It was concluded from the mechanism of SVP hydrolysis that: (i) RaMV VPg is covalently linked with RNAs via a phosphodiester bond; (ii) the 5′-terminal nucleotides are involved in this phosphodiester bond; (iii) the 5′-terminal nucleotides of RaMV virion RNAs seem to be different.

However, several explanations are possible concerning this finding: it resulted from heterogeneity of the 5′-terminal nucleotides inside the family of

Table 1

Amino acid composition (in molar ratios) of RaMV and CPMV VPgs

	RaMV[a]	CPMV[b]
Asp, Asn	4	5
Thr	2	0
Ser	3	1
Glu, Gln	3	3
Pro	3	2
Gly	3	0
Ala	3	2
Val	2	2
Met	1	1
Cys	0	0
Cys_2	0	0
Ile	1	0
Leu	3	1
Tyr	1	2
Phe	1	1
Lys	2	2
His	1	0
Arg	2	5
Trp	1[c]	1
Total	36	28

[a] Mean value from 4 analyses. Ala and Gly (Gly in particular) content may be overestimated because of the decomposition of VPg-bound nucleotide residue

[b] Deduced from the primary structure of B-RNA [6,10]

[c] Estimated from the percentage of destruction in 6 N HCl-0.5% β-mercaptoethanol

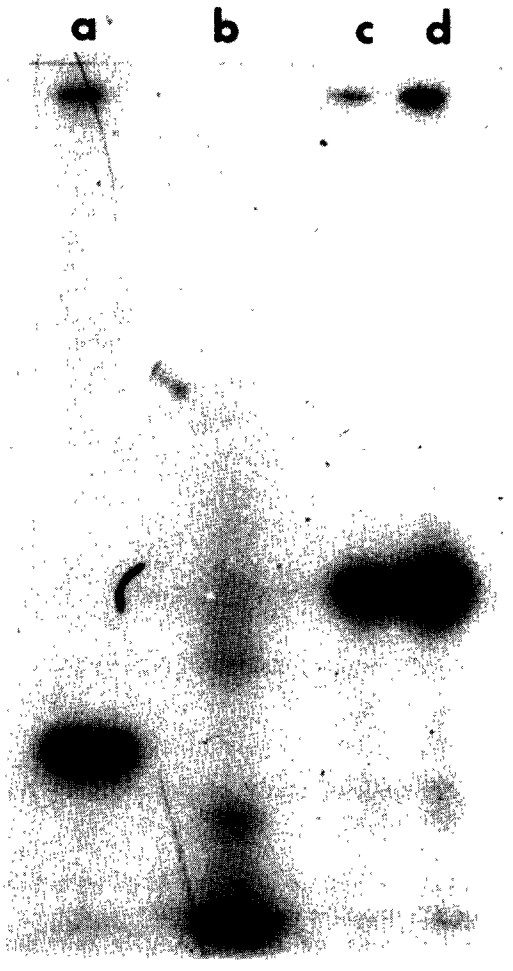

Fig.3. Electrophoresis of RaMV virion RNAs treated with RNases (18% polyacrylamide gel, Laemmli-7 M urea system). a, with RNases A + T_1 + T_2; b, with RNases and then with proteinase K; c,d, B-RNA-^{125}I-VPg and M-RNA-^{125}I-VPg treated with RNase T_1, respectively.

B- and/or M-RNAs. There is yet another possibility: the conditions for complete hydrolysis of EMC RNA-VPg by RNases we used [14] are somewhat different from those of RaMV RNAs-VPg. In this case 5′-end dinucleotide(s) of RaMV RNAs could be protected from hydrolysis by RaMV VPg, which is larger than that of EMC. We hope to understand this problem by sequencing B- and M-5′-end T_1-oligonucleotides. It is noteworthy that the heterogeneity of the very 5′-ends of cowpea mosaic virus RF RNAs has been recently reported [12].

Only *O*-phosphoserine was found by two-dimensional TL electrophoresis in the acid hydrolysates of VPg-[^{32}P]pNp or VPg-[^{32}P]pN produced from RaMV virion RNAs (fig.5). We inferred that RaMV VPg is linked with virion RNAs via a phosphodiester bond between a serine residue of VPg and the 5′-terminal nucleotides.

The resemblance of the genome strategy and VPg structure of como- and picornaviruses was recently discussed [3,4]. However, we can see that different amino acid residues are involved in the formation of the unit of linkage between VPg and the viral genomes. The actual reason for this variety remains to be found.

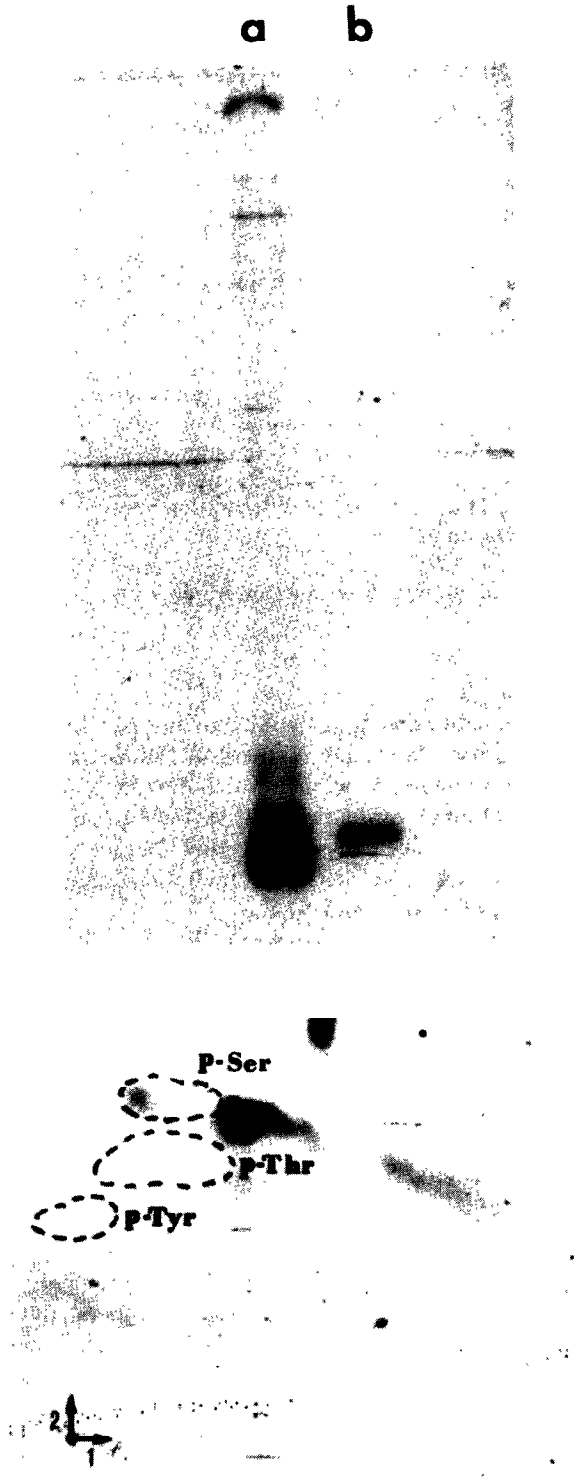

Fig.5. Two-dimensional electrophoresis [13] of VPg-[^{32}P]pN hydrolyzed with HCl/CF_3COOH (2:1, v/v)-1% β-mercaptoethanol at 160°C for 20 min.

Fig.4. Electrophoresis of ^{125}I-VPg-pNp and VPg-[^{32}P]pNp (15% polyacrylamide gel, Laemmli system).

When this paper was in preparation we learned that a serine residue of CPMV VPg appears to be involved in a linkage with virion RNAs [15].

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