JOURNAL OF VIROLOGY, Sept. 1982, p. 1151–1154
0022-538X/82/091151-04$02.00/0

Vol. 43, No. 3

Cowpea Mosaic Virus-Encoded Protease Does Not Recognize Primary Translation Products of M RNAs from Other Comoviruses

ROB GOLDBACH* AND JETTE KRIJT

Department of Molecular Biology, Agricultural University, 6703 BC Wageningen, The Netherlands

Received 29 March 1982/Accepted 27 May 1982

The protease encoded by the large (B) RNA segment of cowpea mosaic virus was tested for its ability to recognize the in vitro translation products of the small (M) RNA segment from the comoviruses squash mosaic virus, red clover mottle virus, and cowpea severe mosaic virus (CPsMV, strains Dg and Ark), and from the nepovirus tomato black ring virus. Like M RNA from cowpea mosaic virus, the M RNAs from squash mosaic virus, red clover mottle virus, CPsMV-Dg, and CPsMV-Ark were all translated into two large polypeptides with apparent molecular weights which were different for each virus and even for the two CPsMV strains. Neither the in vitro products from squash mosaic virus, red clover mottle virus, and CPsMV M RNAs nor the in vitro product from tomato black ring virus RNA-2 were processed by the cowpea mosaic virus-encoded protease, indicating that the activity of this enzyme is highly specific.

Comoviruses have genomes consisting of a large (B) and a small (M) RNA segment, which are separately encapsidated (2, 9, 17). An increasing amount of evidence has been published that B RNA (molecular weight, 2.02×10^6 [13]) of cowpea mosaic virus (CPMV), the type member of the comovirus group, is translated in vivo into a 200-kilodalton (200 K) polyprotein which is proteolytically cleaved into at least eight different polypeptides with sizes of 170 K, 110 K, 87 K, 84 K, 60 K, 58 K, 32 K, and 4 K (6, 7, 14). The 4 K polypeptide represents the genome-linked protein VPg, attached to the 5′ ends of both B and M RNA (3, 15, 16), and is probably directly derived from the 60 K polypeptide (6, 18). M RNA [molecular weight, 1.37×10^6 [13]) of CPMV has been shown to encode the two (37 K and 23 K) capsid proteins (designated VP37 and VP23, respectively), but the expression of this RNA in vivo has not yet been fully elucidated (4). In both wheat germ extract and rabbit reticylocyte lysate, M RNA of CPMV is translated into two overlapping polypeptides of 95 K and 105 K, which are produced from two initiation sites (4, 7, 11). The 95 K and 105 K in vitro polypeptides are efficiently cleaved by a protease encoded by B RNA in vivo, resulting in polypeptides of 60 K, 58 K, and 48 K (4). Since the 60 K cleavage product contains the sequences of both capsid proteins it has been proposed that the 95 K or 105 K polypeptides or both are also produced in vivo, and that the B RNA-encoded protease plays a key role in the release of the two capsid proteins from these polypeptides (4). The studies reported here were undertaken to determine the specificity of the CPMV-encoded protease, i.e., whether it is able to recognize the in vitro translation products of M RNAs from other comoviruses.

The purification of comovirus RNAs, translation in wheat germ extract and rabbit reticulocyte lysate, preparation of proteolytically active extracts from CPMV-infected protoplasts, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis have been described (4, 7, 14). The M RNAs from the comoviruses CPMV (strain Sb), squash mosaic virus (SqMV; American Type Culture Collection no. PV-36), red clover mottle virus (RCMV), cowpea severe mosaic virus (CPsMV) strains Ark and Dg, and RNA-2 from the nepovirus tomato black ring virus (TBRV) (strain A) were translated in rabbit reticulocyte lysate and wheat germ extract, using [^{35}S]methionine as the radioactive amino acid. The products obtained were compared by electrophoresis in 12.5% polyacrylamide gels. The analyses indicate that translation of M RNA into two large polypeptides is a common feature of comoviruses (Fig. 1 and 2). In the reticulocyte lysate (Fig. 1) several smaller polypeptides were also synthesized, but in the wheat germ system (Fig. 2) the M RNAs from SqMV, CPMV, RCMV (not shown), CPsMV-Ark (not shown), and CPsMV-Dg were all exclusively translated into two large polypeptides with sizes ranging from 90 K to 110 K. The polypeptide band of approximately 50 K present in all lanes of Fig. 1 represents an endogenous product of the reticu-

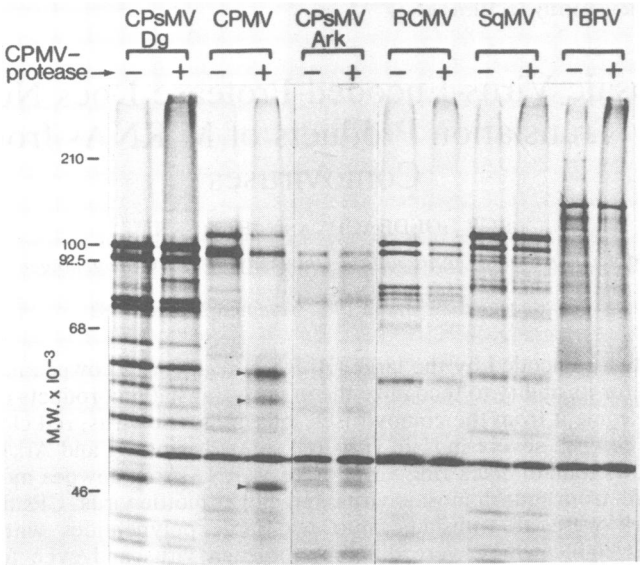

FIG. 1. Analysis of the in vitro products of various comovirus M RNAs and of TBRV RNA-2. RNAs were translated in rabbit reticulocyte lysate, using [^{35}S]methionine as the radioactive amino acid, as described previously (7, 12). Portions of the products were left untreated (−) or added to an equal volume of 30,000 × g supernatant fraction from CPMV-infected protoplasts (+). This fraction has been shown to contain the B RNA-encoded protease (for details see reference 4). After 1 h of incubation at 30°C the samples were analyzed in a 12.5% polyacrylamide gel as previously described (10, 14). The molecular weights indicated at the left side of the gel refer to the position of the marker proteins used, which included myosin (210,000), phosphorylase b (100,000 and 92,500), bovine serum albumin (68,000), ovalbumin (46,000), and carbonic anhydrase (30,000). The TBRV RNA-2 preparation used contained trace amounts of TBRV RNA-1, resulting in the synthesis of some 220 K polypeptide (5). The arrows in the gel indicate the proteolytic cleavage products of CPMV M RNA.

locyte lysate batch used and is presumably the result of a ribosome-independent process (12). For CPMV it has been shown that the two M RNA-encoded 95 K and 105 K polypeptides have overlapping amino acid sequences and are the result of two initiation sites (4, 11). The results presented here suggest that two in vitro-active initiation sites on M RNA are a common feature of comoviruses. Table 1 summarizes the average sizes of the M RNA in vitro translation products from the comoviruses tested, each calculated from six different translation experiments. No significant differences in size were found between the products made in reticulocyte lysate and the products made in wheat germ extract. Hiebert and Purcifull (8) and Beier et al. (1) calculated slightly different sizes for the products of SqMV M RNA (i.e., 112 K and 105 K) and CPsMV-Dg M RNA (108 K and 98 K), using different gel electrophoresis conditions and different molecular weight markers. To test whether the protease encoded by B RNA of CPMV was capable of recognizing the M RNA-encoded polypeptides from other comoviruses or the capsid protein-containing 140 K product of TBRV RNA-2 (5), the various in vitro products were mixed with an equal volume of 30,000 × g supernatant fraction from CPMV-infected protoplasts and incubated for 1 h at 30°C. This fraction is known to contain the viral protease activity (4). After incubation the mixtures were electrophoresed along with the untreated in vitro products (cf. Fig. 1 and 2, lanes indicated with + and −, respectively). It is clear that only the in vitro products of CPMV M RNA are cleaved by the CPMV protease, giving the 60 K, 58 K, and

TABLE 1. Polypeptides translated in vitro from M RNAs of various comoviruses

Virus	Polypeptide size[a] (K)
SqMV	107, 100
CPMV	105, 95
RCMV	102, 96
CPsMV-Dg	100, 93
CPsMV-Ark	95, 91

[a] Calculated from six different translation experiments. Polypeptides were electrophoresed in 12.5% polyacrylamide gels, and their sizes were estimated with [^{14}C]methylated myosin (210 K), phosphorylase b (100 K and 92.5 K), and bovine serum albumin (68 K) as size markers.

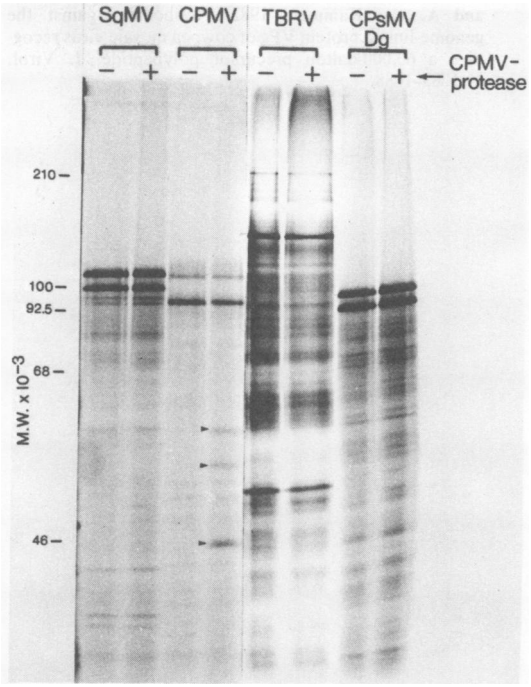

FIG. 2. Analysis of the in vitro products of various comovirus M RNAs and TBRV RNA-2, as obtained in wheat germ cell-free system. RNAs were translated in wheat germ extract, using [^{35}S]methionine as the radioactive amino acid, as described previously (7). Portions of the translation products obtained were left untreated (−) or added to an equal volume of 30,000 × *g* supernatant fraction of CPMV-infected protoplasts (+). After 1 h of incubation at 30°C the samples were analyzed in a 12.5% polyacrylamide gel. The numbers at the left side refer to the molecular weights of the marker proteins (see legend of Fig. 1) used. The TBRV RNA-2 preparation contained trace amounts of TBRV RNA-1, which resulted in the synthesis of some 220 K polypeptide. The arrows in the gel indicate the proteolytic cleavage products of CPMV M RNA.

48 K cleavage products (arrows in Fig. 1 and 2) described in detail elsewhere (4). Neither the products from the various other comovirus M RNAs nor the 140 K TBRV RNA-2 product is recognized by this protease. With CPsMV-Dg sometimes a new polypeptide of approximately 87 K was found after incubation with CPMV protease (cf. Fig. 1 and 2). Since this polypeptide was not produced when the CPsMV-Dg in vitro products were incubated with extract from CPsMV-infected protoplasts (instead, three cleavage products were then produced, with sizes of 58 K, 55 K, and 45 K [results not shown]), it is probably the result of some fortuitous cut by the CPMV protease. Our conclusion is that the activity of the CPMV-encoded protease is highly specific and not able to recognize the primary translation products of M RNAs from other comoviruses.

We thank Ernest Hiebert, Jeffrey Davies, Hildburg Beier, Alan Brund, and Mike Mayo for the gift of SqMV M RNA, RCMV M RNA, CPsMV-Dg, CPsMV-Ark, and TBRV RNA-2, respectively; Peter Blackburn for the gift of human placental RNase inhibitor; Hugh Pelham and Richard Jackson for the gift of rabbit reticulocyte lysate; and Marie-José van Neerven for typing the manuscript.

This work was supported by the Netherlands Foundation for Chemical Research, with financial aid from the Netherlands Organization for the Advancement of Pure Research.

LITERATURE CITED

1. **Beier, H., O. G. Issinger, M. Deuschle, and K. W. Mundry.** 1981. Translation of the RNA of cowpea severe mosaic virus in vitro and in cowpea protoplasts. J. Gen. Virol. **54**:379–390.
2. **Bruening, G.** 1977. Plant covirus systems: two-component systems, p. 55–141. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 11. Plenum Publishing Corp., New York.
3. **Daubert, S. D., G. Bruening, and R. C. Najarian.** 1978. Protein bound to the genome RNAs of cowpea mosaic virus. Eur. J. Biochem. **92**:45–51.
4. **Franssen, H., R. Goldbach, M. Broekhuijsen, M. Moerman, and A. van Kammen.** 1982. Expression of middle-component RNA of cowpea mosaic virus: in vitro generation of a precursor to both capsid proteins by a bottom-component RNA-encoded protease from infected cells. J. Virol. **41**:8–17.
5. **Fritsch, C., M. A. Mayo, and A. F. Murant.** 1980. Translation products of genome and satellite RNAs of Tomato Black Ring Virus. J. Gen. Virol. **46**:381–389.
6. **Goldbach, R., G. Rezelman, P. Zabel, and A. van Kammen.** 1982. Expression of the bottom-component RNA of cowpea mosaic virus: evidence that the 60-kilodalton VPg precursor is cleaved into single VPg and a 58-kilodalton polypeptide. J. Virol. **42**:630–635.
7. **Goldbach, R. W., J. G. Schilthuis, and G. Rezelman.** 1981. Comparison of in vivo and in vitro translation of cowpea mosaic virus RNAs. Biochem. Biophys. Res. Commun. **99**:89–94.
8. **Hiebert, E., and D. E. Purcifull.** 1981. Mapping of the two coat protein genes on the middle RNA component of squash mosaic virus (comovirus group). Virology **113**:630–636.
9. **Jaspars, E. M. J.** 1974. Plant viruses with a multipartite genome. Adv. Virus Res. **19**:37–149.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Pelham, H. R. B.** 1979. Synthesis and proteolytic processing of cowpea mosaic virus proteins in reticulocyte lysates. Virology **96**:463–477.
12. **Pelham, H. R. B., and R. J. Jackson.** 1976. An efficient mRNA-dependent translation system from reticulocyte lysates. Eur. J. Biochem. **67**:247–256.
13. **Reijnders, L., A. M. J. Aalbers, A. van Kammen, and R. W. J. Thuring.** 1974. Molecular weights of plant viral RNAs determined by gel electrophoresis under denaturing conditions. Virology **60**:515–521.
14. **Rezelman, G., R. Goldbach, and A. van Kammen.** 1980. Expression of bottom component RNA of cowpea mosaic virus in cowpea protoplasts. J. Virol. **36**:366–373.
15. **Stanley, J., R. Goldbach, and A. van Kammen.** 1980. The genome-linked protein of cowpea mosaic virus is coded by RNA from the bottom component. Virology **106**:180–182.
16. **Stanley, J., P. Rottier, J. W. Davies, P. Zabel, and A. van Kammen.** 1978. A protein linked to the 5′ termini of both RNA components of the cowpea mosaic virus genome.

Nucleic Acids Res. **5**:4505–4522.
17. **Van Kammen, A.** 1972. Plant viruses with a divided genome. Annu. Rev. Phytopathol. **10**:125–150.
18. **Zabel, P., M. Moerman, F. van Straaten, R. Goldbach, and A. van Kammen.** 1982. Antibodies against the genome-linked protein VPg of cowpea mosaic virus recognize a 60,000-dalton precursor polypeptide. J. Virol. **41**:1083–1088.