Characterization and Translation Studies of Potato Virus S RNA

J. Monis and G. A. de Zoeten

Graduate student and professor, respectively, Department of Plant Pathology, University of Wisconsin-Madison, Madison 53706. Present address of the first and second authors are: Department of Pharmacology, Southwestern Medical Center, University of Texas, Dallas 75235; Department of Botany and Plant Pathology, University of Michigan, East Lansing 48823, respectively.

We wish to thank Dr. S. Waterman for helpful discussions on the in vitro translation studies; Dr. S. L. Loesch-Fries for introducing us to the protoplast inoculation technique, for helpful discussions, and for reviewing this manuscript; Dr. J. P. Helgeson for the use of his laboratory facilities for the isolation of protoplasts and for reviewing this manuscript; S. A. Demler for reviewing this manuscript; and Dr. S. A. Slack for providing PVS antisera. This research was supported by the College of Agriculture and Life Sciences of the University of Wisconsin-Madison and by Hatch Act funds 142-2799.

Accepted for publication 13 September 1989 (submitted for electronic processing).

ABSTRACT

Monis, J., and de Zoeten, G. A. 1990. Characterization and translation studies of potato virus S RNA. Phytopathology 80:441-445.

The M_r of the Andean (An) strain of potato virus S (PVS) RNA was determined to be 2.4×10^6 (7.5 kilobases [kb]) when assayed by electrophoresis under denaturing conditions. Oligo d(T) cellulose chromatography and direct RNA sequencing demonstrated that viral RNA is polyadenylated. In vitro translation in rabbit reticulocyte lysates indicates that four major protein products of 140K, 123K, 104K, and 34K are produced by PVS-An RNA. Antisera specific to the Andean and type strains of PVS immunoprecipitated a major protein product of 34K. Infection of potato protoplasts indicated the production of a polypeptide of 34K, which was not detected in mock inoculated protoplasts and reacted specifically with PVS-An antisera in immunoprecipitation experiments.

Potato virus S (PVS), a member of the carlavirus group, is one of the most common viruses infecting potato (28). Carlavirus particles are about 610–700 nm in length, composed of a single coat protein subunit with an M_r of about 33×10^3 and one molecule of positive-sense-single-stranded RNA with an M_r between 2.3 and 2.6×10^6 (7–7.8 kilobases [kb]) (16). Although the virus is virtually symptomless in most of the common potato cultivars, reduction in yield as high as 10–20% has been reported (28). Symptoms when present are slight deepening of the veins, rugosity of leaves, and stunting (3). The Andean (An) strain of PVS (PVS-An) is transmitted by aphids in a nonpersistent manner; however, aphids do not transmit the type (Ty) strain (25). Studies suggest that the virus does not require the presence of a helper component protein for aphid transmission as do members of the potyviruses (25).

There is little information on the genome organization and expression of the carlavirus group. Translation of potato virus M, a member of the carlavirus group, in a wheat germ cell-free system indicated the production of a major protein of 190K (26). None of the minor protein products corresponded in size to the coat protein subunit. None of the mechanisms of genome expression used by other plant viral groups have yet been proven for the carlavirus group. These mechanisms include: multipartite RNA genomes (i.e., alfalfa mosaic virus group, bromoviruses), subgenomic mRNAs (i.e., bromoviruses, tobamoviruses), proteolytic cleavage of a polyprotein (i.e., comoviruses, potyviruses) and readthrough of leaky termination codons (i.e., tymoviruses, tobamoviruses) (6,10). The long-range objective of our study is to identify the genetic basis of the difference between PVS-An and PVS-Ty. In this paper we report data on the isolation and characterization of PVS-An RNA. Translation of the RNA in reticulocyte lysates and infection of potato protoplasts was used to identify viral specific polypeptides.

MATERIALS AND METHODS

Virus isolate. PVS-An was isolated by sequential single-lesion transfers in *Chenopodium quinoa* Willd. from the potato cultivar Red La Soda (25).

Virus purification and RNA extraction. *C. quinoa* plants with six to eight true leaves were inoculated with PVS-An. Characteristic systemic symptoms developed in 2–3 wk. PVS-An infected tissue was harvested and frozen at −20 C. Frozen infected tissue was homogenized in extraction buffer (0.5 M sodium borate, pH 8.2, 1% sodium sulfite, 0.1% β-mercaptoethanol) at a ratio of 1 g of tissue to 3 ml of buffer. The homogenate was emulsified with one volume (w/v) of cold chloroform, and centrifuged at 8,000 rpm (Sorvall GSA rotor, Du Pont Co., Wilmington, DE) for 10 min. The aqueous phase was filtered through two layers of Miracloth (Cal Biochem, La Jolla, CA), and the virus was precipitated with polyethylene glycol (PEG) (6%, w/v, 0.3 M NaCl). The virus was suspended in one-third the original volume of 0.05 M sodium borate, pH 8.2, and was treated with 0.5% Triton X-100 for 0.5 hr and again precipitated with PEG (4%, w/v, 0.3 M NaCl) and resuspended in 0.05 M sodium borate, pH 8.2. The virus preparation was further purified in a CsCl density gradient (average density = 1.324 g/cm^3). RNA was isolated from virions by the guanidine thiocyanate-LiCl procedure described by Cathala et al (5). Yields of virus and RNA were about 9 mg and 150 μg per 100 g of fresh tissue, respectively.

Determination of relative molecular mass of viral RNA. Genomic PVS-An RNA was denatured with formaldehyde and formamide and subjected to electrophoresis in 1% agarose gels (14). The following viral RNAs were used as molecular weight markers:brome mosaic virus (BMV) (1.09 [3.3 kb], 0.99 [3.1 kb], 0.75 [2.1 kb], 0.28×10^6 [0.7 kb]) (1,2), potato virus Y (PVY) (2.9×10^6 [9 kb]) (7), tobacco mosaic virus (TMV) (2×10^6 [6.4 kb]) (9). The molecular weight of PVS-An RNA was estimated by linear regression analysis of the distance migrated in the gel and the log of the molecular weight of the RNA markers.

Three-prime-end characterization. To determine if PVS-An RNA is polyadenylated, PVS-An RNA was bound to oligo (dT)-cellulose (BRL Inc., Gaithersburg, MD) by batch hybridization (1 hr at room temperature, shaken once a minute). The oligo (dT)-cellulose with bound RNA was loaded onto a column and washed with 10–15 column volumes of 20 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.1% SDS. Bound RNA was eluted with 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 0.05% SDS. Fractions of 300 μl were collected and scanned in a spectrophotometer at 220–320 nm to

determine the RNA content. Fractions containing RNA were ethanol precipitated. To determine if a poly (A) tract was located at the 3′ end of the viral RNA, PVS-An RNA was 3′ end-labeled with (^{32}P)-pCp (3,000 Ci/mmol) (Amersham Corp., Arlington Heights, IL) using T4 RNA ligase (8). The labeled PVS-An RNA was incubated for 4 hr at 37 C with RNase T2 (in 0.25 M sodium citrate, pH 5.0). Treated RNA and 3′-mononucleotide standards were spotted on a PEI cellulose plate (Sigma Chemical Co., St. Louis, MO) and chromatographed in 1 M LiCl. The plate was dried, markers were located by illumination with UV light, and PVS-An RNA cleavage product was detected by autoradiography. Labeled PVS-An RNA was also digested with the following base specific RNases: T1, *Phy* M, CL3, *B. cereus,* and U2. The conditions for reactions were as follows: 25 mM sodium citrate, pH 5.0, 7 M urea, and 1 mM EDTA for RNases M and T1; 25 mM sodium citrate, pH 3.0, 7 M urea, and 1 mM EDTA for RNase U2; 10 mM sodium phosphate, pH 6.5, and 1 mM EDTA for RNases *B. cereus* and CL3. All reactions were incubated for 15 min, at 55 C for RNases *Phy* M, U2, *B. cereus* and T1 and at 37 C for RNase CL3. The digestion products were separated by electrophoresis in 25% polyacrylamide, 10 M urea in 1× TBE (89 mM Tris-borate, 89 mM boric acid, and 2 mM EDTA). Base specific cleavage products were located by autoradiography in comparison to a ladder generated by limited base hydrolysis.

Five-prime-end characterization. To determine if PVS-An RNA has a viral protein linked to its 5′ end (VPg), we attempted to label the RNA with ^{125}I using a method modified by Rekosh et al from Bolton and Hunter (20). The reaction consisted of 42 μg of PVS-An RNA in a 0.1 M sodium borate, pH 8.0, 1 mM EDTA, 1 mM phenylmethylsulfonic fluoride (PMSF) solution plus 100 μCi of lyophilized Bolton and Hunter reagent (2,190 Ci/mmol) (ICN Radiochemicals, Irvine, CA). The reaction was incubated on ice for 30 min and then stopped by the addition of 200 μl of 0.2 M glycine in 0.1 M sodium borate and incubated on ice for 20 additional minutes. The RNA was separated from unincorporated ^{125}I by Sephadex G-50 chromatography (Pharmacia-LKB Biotechnology, Piscataway, NJ). The preparation was assayed by electrophoresis (0.5% agarose), dried, and autoradiographed.

In vitro translation in rabbit reticulocyte lysates. PVS-An RNA was used to direct the synthesis of polypeptides in rabbit reticulocyte lysates (19). The in vitro translation reactions consisted of 35 μl of nuclease treated lysate (Promega Biotec, Madison, WI) at a final concentration of 20 mM Tris-HCl, pH 8.2, 50 μM potassium acetate, 500 μM magnesium acetate, 50 μg/ml of calf liver tRNA, 20 μM hemin, 2 μM DTT, 9.9 mM creatin phosphate, 50 μg/ml of creatin phosphokinase, 20 μM amino acid mixture (minus methionine), 50 μCi (^{35}S)-methionine (1,200 Ci/mmol) (Amersham Corp., Arlington Heights, IL), 8 μg PVS-An RNA, and 50 units of RNase inhibitor (Promega Biotec), in a 50 μl volume that was incubated for 1 hr at 30 C.

Immunoprecipitation of PVS coat protein. The in vitro translation reaction (30 μl) was mixed with 0.1 volume of 20% SDS and boiled for 2 min, diluted with 10 volumes of 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 2% Triton X-100 (TNET), containing 20 mM methionine. After clarification, PVS-An and PVS-Ty antisera were added to the supernatant and incubated at room temperature for 45 min, followed by the addition of 12 μl of a 10% v/v *Staphylococcus aureus* inactivated cell suspension (12). After 15 min the cells were centrifuged through a sucrose pad (35% in TNET) and washed three times with TNET containing 0.1% SDS and once with 0.12 M Tris-HCl, pH 6.8. The cells were pelleted, resuspended in SDS sample buffer (13), boiled for 2 min to release the bound immunoprecipitates and pelleted by centrifugation. Proteins in the supernatant were analyzed by electrophoresis in polyacrylamide gels.

Gel electrophoresis of proteins. Polypeptides were separated by electrophoresis in 10% and 7–15% gradient sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) (13), followed by fluorography (4). The molecular weight of PVS-An protein products were determined by linear regression of the distance migrated by the protein standards and the log of their molecular weights.

Isolation, inoculation, and incubation of protoplasts. Protoplasts were isolated from aseptically grown *Solanum tuberosum* L. 'Russet Burbank' mesophyll tissue as described by Haberlach et al (11). Freshly prepared protoplasts were inoculated with PVS-An RNA as described by Samac et al (24). Briefly, 3×10^5 protoplasts were pelleted, incubated for 10 sec with 10 μg of PVS-An RNA, 50 units of RNase inhibitor, 100 μl of 40% PEG (1,540 molecular weight)-3 mM $CaCl_2$ (pH 5.5), diluted with 10% mannitol and incubated on ice for 20 min. Mock inoculated samples were treated as described above but RNA was omitted in the inoculation mixture. The inoculated protoplasts were washed with mannitol, resuspended in culture media (29), and incubated at 26 C with constant illumination for 18–48 hr.

To detect the virus specific proteins, the protoplasts were irradiated for 3 min with a germicidal lamp (General Electric Co., Cleveland, OH) immediately before inoculation (22) and incubated in the presence of 82.5 μCi/ml (^{35}S)-methionine (>1,000 Ci/mmol) (Amersham Corp.). Samples of infected protoplasts were collected at different times after inoculation, pelleted, and suspended directly in Laemmli sample buffer and proteins were separated by 10% SDS-PAGE. Immunoprecipitation with antisera specific to PVS-An was performed to detect the production of virus coat protein in vivo. Immunoprecipitations were carried out essentially as described above, except that the initial clarification step was omitted. The viral specific proteins were analyzed by SDS-PAGE as described earlier.

RESULTS

Relative molecular mass of PVS-An viral RNA. PVS RNA isolated from purified virions migrated as a single RNA species

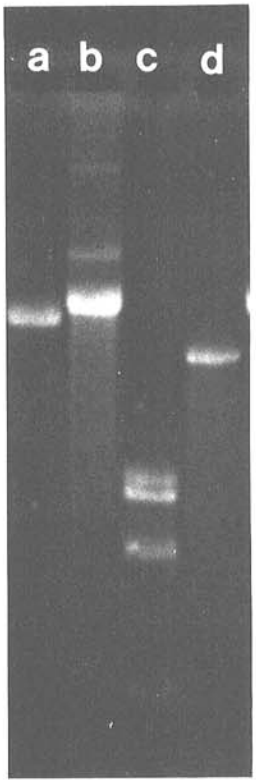

Fig. 1. Agarose gel electrophoresis of PVS-An RNA. Samples were assayed by electrophoresis in a 1% agarose gel at 45 mA constant current for 2.5 hr in 0.01 M sodium phosphate, pH 6.8. The gel was stained with 1 μg/ml ethidium bromide in 0.1 M ammonium acetate for 1 hr and destained in 0.01 M ammonium acetate for 1–2 hr. The RNA (0.5 μg was denatured in 20 mM sodium phosphate, pH 6.8, 50 mM EDTA, 3% formaldehyde, and 25% formamide) and heated at 65 C for 2 min. The samples consisted of the following RNAs: PVS-An (a), PVY (b), BMV (c), and TMV (d).

when subjected to electrophoresis in the presence of formaldehyde and formamide (Fig 1, a). The viral RNA M_r was determined to be 2.4×10^6 (7.5 kb), within the range reported for other carlavirus RNAs (16).

Three-prime-end characterization of PVS-An RNA. Oligo (dT)-cellulose affinity chromatography demonstrated that PVS-An RNA is polyadenylated (data not shown). The poly (A) RNA comigrated with nonfractionated PVS-An RNA when assayed by electrophoresis through agarose gels under nondenaturing and denaturing conditions (data not shown). PVS-An poly (A) RNA was identical to unfractionated PVS-An RNA in in vitro translation; both RNA preparations directed the synthesis of identical protein products in rabbit reticulocyte lysates (data not shown). Analysis of PVS-An RNA labeled at the 3′ end showed that its terminal nucleotide comigrated with the adenosine mononucleotide standard (Fig. 2). Finally, direct RNA sequencing indicated that the poly A tract was at the 3′ end of the RNA (Fig. 3, A and A U).

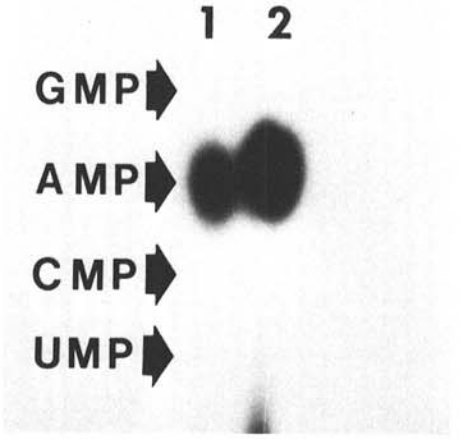

Fig. 2. Thin-layer chromatography of 3′-end-labeled PVS-An RNA. PVS-An RNA was 3′-end-labeled with (^{32}P)-pCp using T4 RNA ligase. The labeled RNA was digested with RNase T2 in 0.25 M sodium citrate, pH 5.0. Treated RNA (0.5 and 1.0 μl, 1 and 2, respectively) and mononucleotide standards were spotted on a PEI cellulose plate, developed in 1 M LiCl and autoradiographed. The arrows indicate the location of the mononucleotide standards.

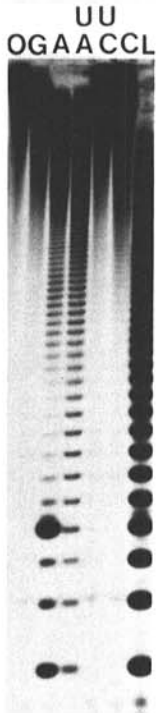

Fig. 3. Direct RNA sequencing of PVS-An RNA. PVS-An RNA labeled with (^{32}P)-pCp was treated as follows: no treatment (O), RNase T1 (G), RNase U2 (A), RNase *Phy* M (A U), RNase *B. cereus* (C U), RNase CL3 (C), and alkaline hydrolysis of RNA to produce a ladder of reference (L), and assayed by electrophoresis in 25% acrylamide gels in 1× TBE (89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA, pH 8.0), containing 10 mM urea and autoradiographed. Lanes A and A U show the poly (A) tract.

Five-prime-end characterization. PVS-An RNA was not labeled with ^{125}I when the Bolton and Hunter reagent was used, suggesting that PVS-An RNA does not have a VPg linked to its genome (data not shown). In the same experiment, pFSC1 and pFSC2 (two mitochondrial plasmids in *Fusarium solani* f. sp. *cucurbitae* that have terminal proteins) (23) were labeled with ^{125}I, but TMV RNA, used as a negative control, was not labeled.

In vitro translation. The in vitro translation of PVS-An RNA in rabbit reticulocyte lysates stimulated a 30–40-fold higher incorporation of (^{35}S)-methionine into proteins than the endogenous controls. Four major polypeptides were produced by PVS-An (Fig. 4, h and i). Initially, it was estimated that the sizes of the products were 124K, 112K, 98K, and 36K (17) when assayed by 10% PAGE. After analysis of the products by 7–15% gradient SDS-PAGE (including a 200K molecular weight marker in addition to the markers used in the 10% PAGE assays) the sizes of the products were estimated to be 140K, 123K, 104K, and 34K (Fig. 4, h and i). When the in vitro translation mixtures were reacted with antisera specific for the Andean and type strains of PVS, a major protein product of 34K was observed (Fig. 4, d–f). This product comigrated with a product in the total in vitro translation products (Fig. 4, h and i). The molecular weight of this protein was similar in size to the coat protein subunit reported for other carlaviruses (27). No major protein product was immunoprecipitated by preimmune serum (Fig. 4, g). To determine

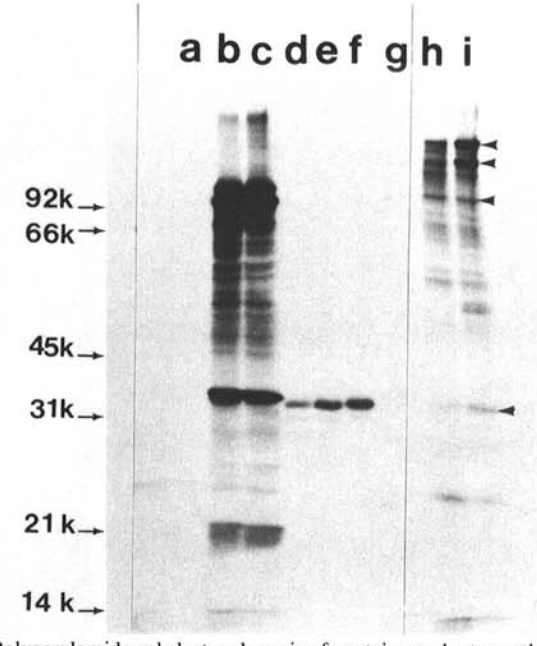

Fig. 4. Polyacrylamide gel electrophoresis of protein products synthesized in rabbit reticulocyte lysates. Protein products were separated by 10% SDS-PAGE and detected by fluorography. Molecular weight markers used to estimate the size of the protein products were: lysozyme (14.4K), soybean trypsin inhibitor (21.5K), carbonic anhydrase (31K), ovalbumin (45K), bovine serum albumin (66.2K), phosphorylase B (92.5K), and myosin (H chain) (200K) and the in vitro translation products of BMV RNA (110K, 105K, 35K, and 20K) (b and c, 30 and 60 min, respectively). Endogenous RNA: no RNA added to the system (a). Immunoprecipitation of PVS-An translation products with a 1:10 dilution of PVS-An antisera (d), undiluted PVS-An antisera (e), undiluted PVS-Ty antisera (f), preimmune serum (g), PVS-An in vitro translation products in rabbit reticulocyte lysates (h and i, 30 and 60 min, respectively). Arrows indicate the major protein products of PVS-An RNA (140K, 123K, 104K, and 34K).

if PVS-An RNA produces a large molecular weight polyprotein that is subsequently cleaved, pulse chase experiments were carried out. Pulses of 10 sec, 20 sec, 30 sec, 2 min, and 30 min with (^{35}S)-methionine, were followed by a 60-min chase with 1,000-fold excess of cold methionine. Coat protein was immunoprecipitated by PVS-An antisera as early as 10 sec into the experiment (Fig. 5). Appearance of the different polypeptides was not time dependent. No high molecular weight precursor of the in vitro products was detected in this experiment or when soybean trypsin inhibitor and PMSF (50 μg/ml and 15 μg/ml, respectively) were incubated with the in vitro translation reactions (data not shown).

Infection of protoplasts and synthesis of viral specific proteins. The incubation of mock inoculated and PVS-An infected protoplasts in the presence of (^{35}S)-methionine permitted the detection of a polypeptide of 34K (Fig. 6, e and j), which was not detected in mock inoculated protoplasts (Fig. 6, f and i). This polypeptide was specifically immunoprecipitated by PVS-An antisera (Fig. 6, c and g). The 34K protein was the only protein detected in a time course experiment from 2 to 48 hr after infection in which samples were taken at 4-hr intervals (data not shown). Irradiation with a germicidal lamp decreased the synthesis of background proteins (Fig. 6, i and j) as compared with the nonirradiated but still did not permit the detection of the larger virus-specific proteins (Fig. 6, e and f).

DISCUSSION

In this paper we present data on the characterization of PVS-An RNA. An M_r of 2.4×10^6 (7.5 kb) for PVS-An genomic RNA is within the range reported for other carlavirus RNAs (16). PVS-An RNA was found to be polyadenylated, as is PVM, another member of the carlavirus group (27). The Bolton and Hunter reagent failed to reveal any evidence for a VPg at the 5′ end of PVS-An.

PVS-An RNA directed the synthesis of four major translational products. The 34K product was shown to be the viral coat protein subunit based on its comigration with authentic PVS-An coat protein and its specific immunoprecipitation with PVS-An antisera. These results differ from those reported by Szybiak and Legocki (26) for PVM, in which only a large molecular weight polypeptide of 190K was detected following translation in a wheat germ cell-free system. The larger polypeptides observed in our work (140K, 123K, and 104K) are probably nonstructural and await further characterization. The coding capacity of PVS-An RNA was estimated to be 270K, which is a smaller value than that calculated by adding the molecular weights of the four proteins produced in vitro (400K). We did not detect a high molecular weight protein precursor of the in vitro translation products by pulse chase or protease inhibitor experiments. This suggests a translational strategy that does not involve the synthesis of a polyprotein that is proteolytically processed as described for members of the potyviruses and comoviruses (10). The excess of protein products observed in our translation studies, as compared with the total coding capacity of PVS-An RNA, is more consistent with the production of read through products and subgenomic mRNAs. The presence of a subgenomic mRNA of about 0.65×10^6, coding for the coat protein, has been suggested for PVM (26). We have not observed a small RNA that could code for a 34K polypeptide in ethidium bromide stained agarose gels (Fig. 1). However, when total RNA isolated from infected tissue of *C. quinoa* was assayed by northern blot hybridization two RNA species of approximately 1.0×10^6 and 0.7×10^6 hybridized to a probe specific to PVS-An RNA (18). These RNA species could be considered putative subgenomic RNAs and if translated might code for proteins of $M_r = 110$K and 77K. These small RNA species were not observed by northern blot analysis when RNA isolated from PVS-An virions and healthy tissue supplemented with virions were hybridized to the same probe (18).

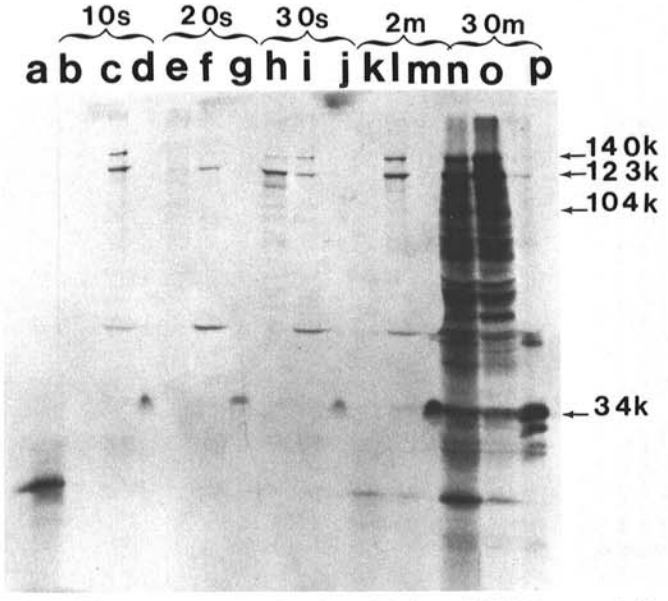

Fig. 5. Time course and pulse chase labeling of PVS-An translation products in a rabbit reticulocyte lysate system. Pulses of 10 sec, 20 sec, 30 sec, 2 min, and 30 min (b, e, h, k, and n, respectively) with (^{35}S)-methionine were followed by a 60-min chase with 1,000-fold excess cold methionine (c, f, i, l, and o, respectively). Immunoprecipitation with antisera specific to PVS-An were performed after each pulse with (^{35}S)-methionine (d, g, j, m, and p, respectively). Lane a represents the products of endogenous RNA. Aliquots at each time (10 μl) were removed and separated by 10% SDS-PAGE and detected by fluorography. The brackets group the three different treatments, pulse, chase, and immunoprecipitation, respectively.

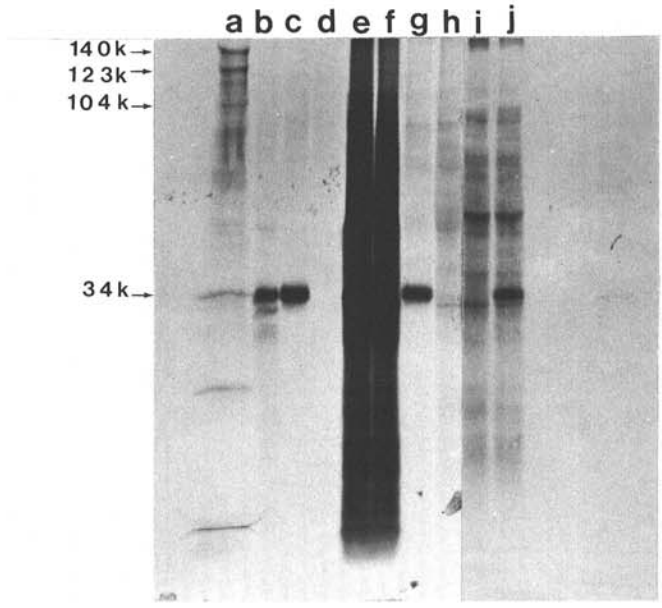

Fig. 6. Fluorograph of labeled proteins separated by 10% SDS-PAGE. Protoplasts were inoculated as described in Materials and Methods and incubated in culture media containing (^{35}S)-methionine for 18 hr at 26 C with constant illumination. The in vitro translation products of PVS RNA (a) and PVS-An products immunoprecipitated with PVS-An specific antisera (b); protein products from nonirradiated protoplasts (c–f): PVS-An inoculated and mock inoculated reacted with PVS-An specific antisera (lanes c and d, respectively); inoculated with PVS-An (lane e), mock inoculated (lane f). Protoplasts were irradiated for 3 min with a germicidal lamp before inoculation (lanes g–j): protoplasts inoculated with PVS-An RNA and mock inoculated reacted to PVS-An antisera (lane g and h, respectively), protoplasts mock-inoculated (i) and inoculated with PVS-An RNA and reacted to PVS-An antisera (j).

The partial sequence of the 3′ end of the Peruvian strain of PVS was reported by MacKenzie et al (15). The sequence contains six open reading frames (ORFs) of 11K, 42K (includes the 33K coat protein), 7K, 12K, 25K, and at least 41K. There are two possible ATG initiation codons for the coat protein cistron coding for proteins of 42K and 33K, respectively. It is not known how the coat protein is produced in vivo. Sequence data obtained for the Peruvian strain of PVS suggests that it may be produced by internal initiation or the translation of a subgenomic RNA (15).

The sequence reported for the Peruvian strain of PVS (15) and PVM (21) suggests that these viruses code for a 25K polypeptide. A protein product of about 25K was observed in our in vitro translation experiments (Fig. 5, a–i). Because a product of that size was observed in all treatments, we considered it to be a background product. If a polypeptide of 25K is produced by PVS-An it was obscured by the background proteins. To detect this polypeptide it will be necessary to produce specific antisera and perform immunoprecipitation experiments.

When potato protoplasts were infected with PVS-An RNA we only detected the production of the viral coat protein, probably because the large M_r proteins are produced in low quantities, very early in infection or are turned over rapidly. These proteins were not detected even at 2 hr postinoculation. When available, antisera specific to the nonstructural proteins would increase the chances of detection of these proteins in vivo. The potato protoplast infection system described in this paper provides a tool for studying different events of the replication of PVS and other potato viruses in a nearly synchronous system. Cloning and sequencing of PVS-An will elucidate the mechanisms of gene expression used by this virus. In the accompanying paper (18) we present results on the cloning and physical mapping of PVS-An complementary DNA representing almost the full length of the viral genome.

LITERATURE CITED

1. Ahlquist, P. G., Dasgupta, R., and Kaesberg, P. 1984. Nucleotide sequence of the brome mosaic virus genome and its implications for viral replication. J. Mol. Biol. 172:369-383.
2. Ahlquist, P. G., Lukow, V., and Kaesberg, P. 1981. Complete nucleotide sequence of brome mosaic virus RNA 3. J. Mol. Biol. 153:23-38.
3. Bagnall, R. H. 1981. Potato virus S. Pages 75-77 in: Compendium of Potato Diseases. W. J. Hooker, ed. American Phytopathological Society, St. Paul, MN. 125 pp.
4. Bonner, W. M., and Laskey, R. D. 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. 46:83-88.
5. Cathala, G., Sauvaret, J. F., Mendez, B., West, B. L., Karin, M., Martial, J. A., and Baxter, J. D. 1983. A method for isolation of intact, translationally active ribonucleic acid. DNA 2:329-335.
6. Davies, J. W., and Hull, R. 1982. Genome expression of plant positive-strand RNA viruses. J. Gen. Virol. 61:1-14.
7. de Bokx, J. A. 1981. Potato Virus Y. No. 242 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 6 pp.
8. England, T. E., Bruce, A. G., and Uhlenbeck, O. C. 1980. Specific labeling of 3′ termini of RNA with T4 RNA ligase. Methods Enzymol. 60:65-74.
9. Goelet, P., Lommonosoff, G. P., Butler, P. J. G., Akem, M. E., Gait, M. J., and Karn, J. 1982. The nucleotide sequence of tobacco mosaic virus RNA. PNAS USA 79:5818-5822.
10. Goldbach, R. W. 1986. Molecular evolution of plant RNA viruses. Annu. Rev. Phytopathol. 24:289-310.
11. Haberlach, G., Cohen, B. A., Rechhert, N. A., Baher, M. A., Towill, L. E., and Helgeson, J. P. 1985. Isolation, culture and regeneration of protoplasts from potato and several *Solanum* species. Plant Sci. 39:67-74.
12. Kesser, S. W. 1976. Cell membrane antigen isolation with staphylococcal protein A-antibody adsorbent. J. Immunol. 117:1482-1490.
13. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) 227:680-685.
14. Lechrach, H., Diamond, D., Wozney, J. M., and Boedker, H. 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry 16:4743-4751.
15. MacKenzie, D., Tremaine, J. H., and Stace-Smith, R. 1989. Organization and interviral homologies of the 3′ terminal portion of potato virus S RNA. J. Gen. Virol. 70:1053-1063.
16. Matthews, R. E. 1979. Classification and nomenclature of viruses. Intervirology 12:129-296.
17. Monis, J., Daniels, S., de Zoeten, G. A., and Slack, S. A. 1987. Characterization of potato virus S (PVS) genomic RNA. Phytopathology (Abstr.) 77:1742.
18. Monis, J., and de Zoeten, G. A. 1990. Molecular cloning and physical mapping of potato virus S complementary DNA. Phytopathology 80:446-450.
19. Pelham, H. R. B., and Jackson, R. 1976. An efficient mRNA dependent translation system from reticulocyte lysates. Eur. J. Biochem. 67:247-256.
20. Rekosh, D. M. K., Russell, W. C., and Bellet, A. J. D. 1977. Identification of a protein linked to the ends of adenovirus DNA. Cell 11:284-295.
21. Rupasov, V. V., Morozov, S. Y., Kanyura, K. V., and Zavried, S. K. 1989. Partial nucleotide sequence of potato virus M shows similarities to potexviruses in gene arrangement and the encoded amino acid sequences. J. Gen. Virol. 70:1861-1869.
22. Sakai, F., and Takebe, I. 1974. Protein synthesis in tobacco mesophyll protoplasts induced by TMV infection. Virology 62:426-433.
23. Samac, D. A. 1989. Characterization of mitochondrial plasmids in *Fusarium solani* f. sp. *cucurbitae*. Ph.D. thesis. University of Wisconsin, Madison. 166 pp.
24. Samac, D. A., Nelson, S. E., and Loesch-Fries, L. S. 1983. Virus protein synthesis in alfalfa mosaic virus infected alfalfa protoplasts. Virology 131:455-462.
25. Slack, S. A. 1983. Identification of an isolate of the andean strain of potato virus S in North America. Plant Dis. 67:786-789.
26. Szybiak, U., and Legocki, A. B. 1981. Translation in vitro of ribonucleic acids from potato virus X, potato virus M and white clover mosaic virus. Acta Biochim. Pol. 26:99-104.
27. Tavantzis, S. 1984. Physicochemical properties of potato virus M. Virology 133:427-430.
28. Wetter, C. 1971. Potato virus S. No. 60 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England.
29. Wood, K. R. 1985. Tissue culture methods in phytopathology. I-Viruses. Pages 193-214 in: Plant Cell Culture. A Practical Approach. R.A. Dixon, ed. IRL Press, Oxford. 236 pp.