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ANALYSIS OF THE COAT PROTEINS OF THE ORDINARY AND ANDEAN STRAINS OF POTATO VIRUS S AND ANTIGENIC COMPARISONS OF CARLAVIRUSES

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Summary. - The coat proteins of both ordinary (PVS^O) and Andean (PVS^A) strains were shown to be similar in molecular weights, peptide maps, amino acid analysis and reaction to a range of carlavirus antiseras.

Key words: PVS; amino acid analysis; peptide mapping; western analysis

Introduction

Potato virus S (PVS), a member of the carlavirus group (Wetter and Milne, 1981), is found in nearly all countries where potatoes are grown (de Bokx, 1970) and is considered as one of the most important filamentous viruses in Europe (Lecoq *et al.*, 1988).

Until recently all isolates in both North America and Europe have shown marked similarities on indicator hosts and potato varieties. Symptoms on potato are, at worst, a mild mosaic and, on indicator plant, *Chenopodium quinoa,* local lesions (de Bokx, 1970; Kaczmarek and de Bokx, 1977). However, in recent years in North America and Europe, isolates of PVS have been detected that induce systemic symptoms in *C. quinoa,* and produce more severe symptoms in various potato cultivars (Rose, 1983; Slack, 1983; Dolby and Jones, 1987). All such isolates have been termed Andean strains (PVS^A), due to their similarity to symptoms induced by an isolate from Peruvian varieties, described by Hinostroza-Orihuela (1973).

In this paper we describe the analysis of the coat proteins of an ordinary strain of PVS from Europe (PVS^O) and an Andean strain (PVS^A) including accurate molecular weight determination, amino acid analysis, peptide mapping and western blot analysis.

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Materials and Methods

Virus purification. All carlaviruses used in this study were purified as previously described by Foster and Mills (1990).

Isolation of viral coat protein. Equal volumes of dissociation buffer (0.02 mol/l NaH_2PO_4 pH 7.0, 2 % SDS (w/v), 0.4 % dithiothreitol (v/v)) and virus suspension were incubated together at 90°C for 2 min. Dissociated protein was either used immediately or stored at room temperature until required.

Cleveland peptide mapping and amino acid analysis of viral coat protein. Dissociated protein was subjected to proteolytic digestion essentially as described by Cleveland (1977). Coat protein suspension (100 μl: approx 200 μg) was incubated at 37°C for 30 min with an assayed volume (5–50 μl) of specific protease (225 μg/ml) in 0.125 mol/l Tris-HCl pH 6.8, 0.5 % SDS, 10 % glycerol. Sample buffer was added to each sample, boiled for 2 min at 90°C, and analysed by polyacrylamide gel electrophoresis (Laemmli, 1970).

Amino acid analysis of viral coat protein was carried out by Nicgene (Belfast, NI). Coat protein (0.2 to 2 mg) was hydrolyzed by heating in the presence of either 6N HCl or 4N methanesulphonic acid containing 0.2 % 3-(2-aminoethyl) indole, both producing similar results.

Polyacrylamide slab gel electrophoresis of proteins. Proteins were separated in 10–12.5 % (w/v) polyacrylamide slab gels (14 cm long, 1.5 mm thick) containing 0.1 % SDS at 50V for 18 hr as described by Laemmli (1970) in Tris/glycine/SDS based electrophoresis buffer. Following electrophoresis, gels were stained for a minimum of 1 hr in 0.25 % coomassie blue in methanol:water:acetic acid (5:5:1) and destained in 5:5:1, minus coomassie blue. Alternatively, proteins were detected by sensitive silver staining procedures using a commercial kit (Amersham) according to manufactures instructions. Unless otherwise stated the folloving were used as molecular weight markers; lysozyme 14,300; B-lactoglobulin 18,400; chymotrypsinogen 25,700; ovalbumin 43,000; bovine serum albumin 68,000; phosphorylase b 97,400; myosin 200,000 (Gibco BRL).

Accurate molecular weight determination by polyacrylamide tube-gel analysis. The molecular weight of viral coat protein was determined by polyacrylamide gel electrophoresis essentially as described by King (1970) using four polyacrylamide gel concentrations (5, 7, 9, 11 %). Different strength gels were cast in 11 cm long, 4.5 mm diameter siliconised glass tubes and run simultaneously in 0.2 mol/l phosphate buffer. Coat protein was prepared as described and loaded in sample buffer with electrophoresis continuing until the bromophenol blue tracking dye had run close to the end of the lowest concentration gel. The bromophenol blue front was marked by wire after alectrophoresis and the gels stained and destained. The mobility of the proteins with respect to the bromophenol blue was calculated. The data were analysed using the equations given by Rodbard and Chrambach (1971) and Frank and Rodbard (1975). The retardation coefficient, KR, for each polypeptide was estimated from slopes of plots of log Rf (electrophoretic mobility) against gel strength (%). The molecular weights of the sample proteins were subsequently determined from a plot of retardation coefficients KR vs molecular weight.

Western blot analysis of proteins. Protein samples were separated on 12.5 % polyacrylamide gels followed by electroblotting onto nitrocellulose pre-soaked in transfer buffer (25 mmol/l Tris-HCl pH 8.3, 192 mmol/l glycine, 20 % methanol) at 60 V (0.22A) for 2–3 hrs in a BioRad Trans Blot Cell with water cooling. The transfer blots were placed in a solution of blocking buffer (5 % dried milk powder in TBS, 0.05 mol/l Tris-HCl pH 7.5, 0.2 mol/l NaCl) and incubated overnight. Filters were washed 5 times in TBS containing 0.05 % Tween-20, then incubated with suitable antiserum (diluted 1:500 in blocking buffer) for 90 min. After several washed with TBS-Tween, bound antibody was detected by incubation with alkaline phosphatase conjugated goat anti-rabbit Ig (Sigma) for 90 min followed by several washed and colour development. The colour reaction was developed with Fast Red (Sigma) (1 mg/ml), 25 mmol/l sodium 1-naphthyl phosphate in 0.033 mol/l Tris-HCl pH 9.5. Reactions were stopped by several washes with distilled water.

Double antibody sandwich (DAS) ELISA. All ELISA work was carried out as described by Clark and Adams (1977) using the double antibody sandwich method. PVS Ig and PVS specific enzyme-conjugated Ig were supplied by Bioreba AG, Switzerland.

Results and Discussion

Analysis of PVS coat protein

Purified virus particles of both ordinary (PVS^{O} A. V.) and Andean (PVS^{A} CE 7260/12) strains were disrupted to yield the viral coat protein. Analysis by polyacrylamide gel electrophoresis in 12.5 % slab gels containing SDS, showed PVS^{A} and PVS^{O} coat proteins to have similar molecular weights of M_r 34,000 and 33,800 respectively (Fig. 1, lanes 1 and 2, Fig. 2, lanes 3 and 4). Trace amounts of a lower M_r species consistently present in both preparations were

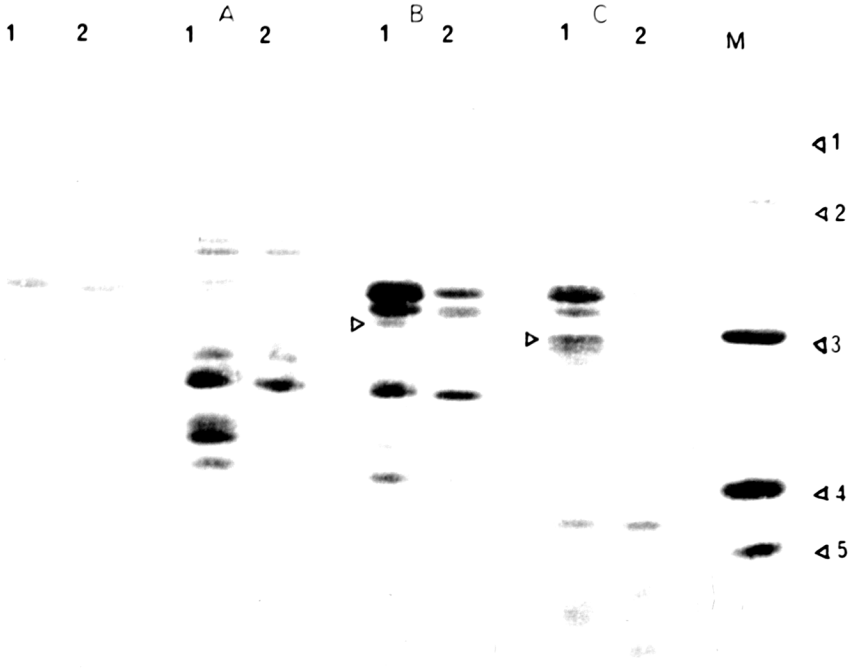


Fig. 1

Cleveland peptide mapping of ordinary and Andean coat proteins

Coomassie blue staining of a 15 % polyacrylamide gel. Lane 1, PVS^{A}; Lane 2, PVS^{O} coat protein.

(A) *Staphyloccocus aureus* V8

(B) Trypsin

(C) Chymotrypsin

(M) Molecular weight markers 1 (68,000), 2 (43,000), 3 (25,700), 4 (18,500), 5 (14,300).

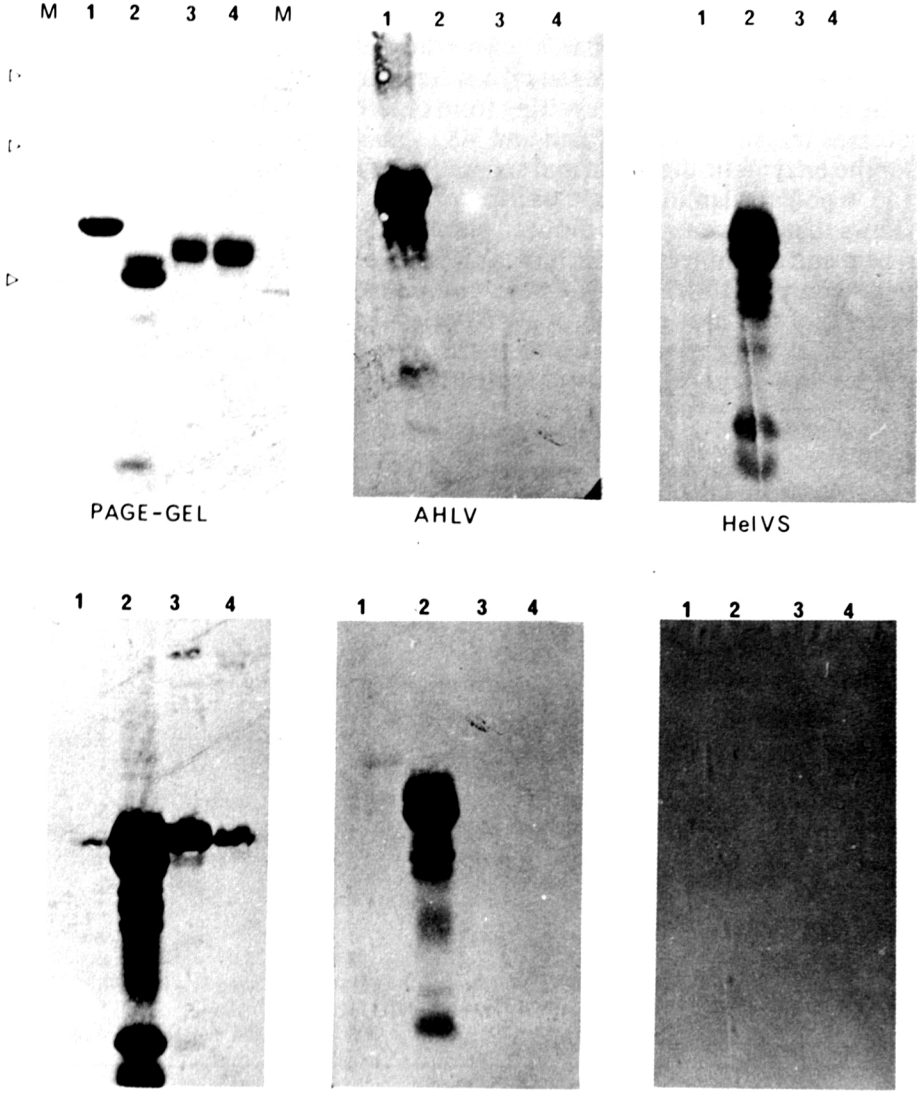
Positions of possible unique fragments are indicated (open arrows)

presumed to be a proteolytic degradation product. Detailed work with potato virus X (PVX) coat protein indicated that the appearance of a second minor protein band was due to action by plant proteases (Koenig *et al.*, 1978). To determine an accurate molecular weight, eliminating any variability of M_r with variation in gel strenght, the coat proteins were analysed at four different gel concentrations and the molecular weights determined as described in *Materials and Methods*. Plotting KR determined in varying gel strengths, against molecular weights of known standards, gave values of M_r 34,000+/-200 for PVS^A and 33,700+/-100 for PVS^O as means from 3 experiments.

The number of peptides resulting from digestion of viral coat proteins by the proteases trypsin, chymotrypsin and V8 (*Staphyloccocus aureus*) was assessed after the enzymatic digestion and separation of the peptides by electrophoresis on 15 % polyacrylamide gels, essentially as described by Cleveland (1977). Fig. 1 shows that each enzyme produced similar patterns for both PVS^O and PVS^A. Trypsin and chymotrypsin did however, induce one additional peptide in PVS^A digests compared with PVS^O. These unique bands may be due to the slightly larger M_r of the coat protein of PVS^A or a slight variation in the amino acid sequence at the cleavage sites of these proteases. The use of these unique peptides as a diagnostic feature is clearly hindered by the close proximity of

Table 1. Amino acid analysis of the PVS^A and PVS^O coat proteins

Amino acid	Tremaine and Goldsack	PVS^A(CE)	PVS^O(Roy)
Asp	16	16	18
Glu	17	19	20
Ser	10	10	10
Gly	9	10	12
His	3	3	4
Arg	11	13	11
Thr	8	6	8
Ala	13	18	16
Pro	11	16	15
Tyr	3	3	3
Val	10	9	10
Meth	6	7	7
Cys	1	1	1
Ile	10	8	8
Leu	9	10	10
Phe	4	5	5
Lys	4	6	4
Total	144	160	162


M 1 2 3 4 M
PAGE-GEL
1 2 3 4
AHLV
1 2 3 4
HelVS
1 2 3 4
1 2 3 4
1 2 3 4

other major peptides and similarity in these regions with the ordinary isolate. These initial results indicate that the coat proteins of PVS^O and PVS^A are highly homologous. This was further confirmed by amino acid analyses of the coat proteins of the two strains (PVS^O ROY and PVS^A CE 7260/12). The results are summarized in Table 1 with the number of residues of each amino acid calculated from picomoles obtained from the analysis assuming the presence of a single cysteine. The results indicate that the two strains are essentially similar with only slight variations, with a maximum difference in values of 2 residues. Indeed both estimates were in close agreement with an amino acid composition for PVS previously reported by Tremaine and Goldsack (1968).

Antigenic comparisons of carlaviruses by Western blot analysis

When equal quanties (mg) of purified PVS^O and PVS^A isolates, determined by spectrophotometry, were serially diluted and tested against PVS antiserum (BioReba AG) by ELISA, similar dilution end points were obtained in all experiments for both strains. Similar results were obtained for both strains when coat proteins were analysed by Western blotting using commercial PVS antiserum (results not presented). However, a number of interesting observations were obtained when the coat proteins of Helenium virus S, American hop latent virus, and the two PVS strains were analysed by Western blotting using a number of carlavirus antisera, kindly provided by D. J. Barbara, East Malling. The results presented in Fig. 2 clearly show that the antiserum to poplar mosaic virus (PMV) was unable to detect any of the coat proteins tested, though was able to detect PMV in infected sap (results not presented). American hop latent (AHLV) antisera exhibited a strong homologus reaction but no cross reactivity with HelVS or PVS coat proteins. A faint cross reaction was obtained with hop mosaic virus (HMV) antisera and AHLV coat protein. Strong cross reactions were obtained between Impatiens latent virus (ILV), HMV and HelVS antisera, with HelVS coat protein and to a lesser extent PVS coat protein. Thus HMV, PVS, ILV and HelVS seem antigenically similar, with AHLV distantly related to HMV but, as with PMV, distinct from the majority of viruses and from each other. Similar observations have previously been reported by Adams and Barbara (1982) based on direct and F $(ab)_2$-based ELISA. The results presented here however, were obtained by Western blot analysis, from polyac-

Fig. 2

Western blot analysis of carlavirus coat proteins

Lane 1, American hop latent virus; lane 2, Helenium virus S; lane 3, potato virus S^O; lane 4, potato virus S^A. (M) molecular weight markers.

PAGE-GEL, coomassie blue stained gel equivalent to the Western blots reacted with:

AHLV, American hop latent virus antisera

HelVS, Helenium virus S antisera

ILV, Impatiens latent virus antisera

HMV, Hop mosaic virus antisera

PMV, Poplar mossaic virus antisera

rylamide gels containing SDS, reflecting homologies at the amino acid level via sequence epitopes.

In conclusion, it appears that the distinct biological properties of Andean and ordinary strains of PVS are not reflected in substantial differences in physicochemical properties. The coat proteins of the two strains were similar in size, amino acid content and peptide maps. This similarity was also reflected in similar responses for the two strains in serological tests, for a wide range of antisera in Western blot analysis, and PVS antisera in ELISA.

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