Identification of Viral Structural Proteins in the Nucleoplasm of Potato Yellow Dwarf Virus-infected Cells

By NA-SHENG LIN,1* YAU-HEIU HSU 2 AND REN-JONG CHIU 2

1Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan 11529 and 2Agriculture Biotechnology Laboratories, National Chung Hsing University, Taichung, Taiwan 40227, Republic of China

(Accepted 22 June 1987)

SUMMARY

Antiserum raised against purified potato yellow dwarf virus (PYDV) reacted specifically with all five viral structural proteins in immunoblots and also with ultrathin sections of infected tobacco cells. In these sections viral proteins were detected with gold-labelled anti-IgG antibodies and gold particles were predominantly located over the nucleoplasm and over PYDV virus particles either at the periphery of nuclei or in the cytoplasm. Control sections of uninfected or healthy cells were not labelled. The results suggest that the nucleoplasm is the only site of accumulation of PYDV structural proteins.

Potato yellow dwarf virus (PYDV) has bacilliform particles and belongs to subgroup II of the rhabdoviruses, particles of which characteristically bud at the inner nuclear membrane and accumulate mainly in the perinuclear space (Peters, 1981; Francki et al., 1981, 1985). Rhabdoviruses in this subgroup cause disorganization of host cell nuclei, such as the reduction or disappearance of chromatin material, swelling of the nucleolus and the appearance of viroplasm-like structures (Martelli & Russo, 1977).

Although the ultrastructure of PYDV-infected tissue has been thoroughly studied (Black et al., 1965; Black, 1970; Francki et al., 1981, 1985), there has been no immunocytochemical study of viral proteins in situ. We have examined the cellular location of viral structural proteins in infected cells by immunogold staining of ultrathin sections.

The Sanguinolata isolate of PYDV (a gift from Dr H. T. Hsu, Beltsville Agricultural Research Center, USDA, Beltsville, Md., U.S.A.) was mechanically inoculated to, and maintained in, Nicotiana rustica or N. benthamiana in a greenhouse kept at between 25 and 30 °C.

PYDV was purified by a procedure similar to that described by Jackson & Christie (1977). Leaves were triturated in extraction buffer (0·1 M-Tris-HCl pH 8·4, 0·001 M-magnesium acetate, 0·04 M-Na2SO3, 0·001 M-MnCl2). After low speed centrifugation of the extract, K2HPO4 and CaCl2 were added to 50 mM each with stirring and the mixture was centrifuged at low speed. The supernatant fluid was then layered on a 5 ml 20% sucrose (w/v) in extraction buffer and centrifuged at 27000 r.p.m. for 45 min in a Beckman type 30 rotor. Virus particles were suspended in maintenance buffer (as extraction buffer but pH 7·5) and further purified by centrifugation in 5 to 30% (w/v) sucrose rate-zonal and 30 to 60% sucrose (w/v) quasi-equilibrium gradients. Purified virus particles were stored in maintenance buffer at -70 °C.

Antiserum against PYDV was produced in BALB/c mice by five weekly intraperitoneal injections of SDS-disrupted virus preparations. Approximately 100 µg protein (estimated from the intensity of Coomassie Brilliant Blue staining of a polyacrylamide gel of a sample) suspended in 0·1% SDS was used in each injection. An equal volume of Freund's complete adjuvant was added to antigen only in the first injection. Sera were collected 4 days after the last injection.
Purified PYDV was disrupted in protein cracking buffer (Lane, 1978) and electrophoresed in a 10% polyacrylamide gel using a discontinuous buffer system (Laemmli, 1970). Proteins in the gel were transferred to nitrocellulose with a Semidry Electroblotter (Sartorius) at a current density of 0.8 mA/cm² for 1 hr (Kyhse-Andersen, 1984). The buffers were 25 mM-Tris/40 mM-6-amino-n-hexanoic acid/20% (v/v) methanol pH 9.4 as a cathode buffer and 300 mM-Tris/20% (v/v) methanol pH 10.4; 25 mM-Tris/20% (v/v) methanol pH 10.4 as the anode buffer.

The protein blot containing transferred proteins was cut into vertical strips. Protein bands were detected by staining with colloidal gold (Moeremans et al., 1985). After extensive washing with 0.01 M-phosphate-buffered saline (PBS) pH 7.2 containing 0.3% (w/v) Tween 20 and 1 mM-EDTA at 37 °C for 30 min, a further wash with PBS-Tween at room temperature and a rinse in excess distilled water, blots were incubated with a stabilized solution of gold particles (15 nm diam.), prepared as described by Horisberger (1979), containing 0.1% Tween 20 in 10 mM-citrate buffer pH 3.0, until a pink colour developed.

For immunolabelling, blot strips were first blocked with 3% bovine serum albumin in PBS (B buffer) for 1 h at 37 °C. Mouse anti-PYDV serum was then added to the blocking solution to a final dilution of 1/500 and strips were incubated for 2 h at room temperature. After washing extensively in PBS-Tween, strips were then blocked with B buffer and subsequently incubated with horseradish peroxidase conjugated to affinity-purified goat anti-mouse IgG (Bio-Rad) for 2 h. The immunoblots were then washed in PBS-Tween, rinsed with distilled water and treated with a peroxidase substrate solution (60 mg of 4-chloro-1-naphthol dissolved in 20 ml methanol, added to 100 ml PBS, containing 40 μl of 30% H₂O₂).

PYDV-infected plants were kept dark overnight before sampling. Two weeks after inoculation, young leaf tissues showing systemic symptoms were cut into small pieces and vacuum-infiltrated with 0.1 M-phosphate-citrate buffer pH 7.2 (PC buffer) and chilled for 2 h at 4 °C (Langenberg, 1979). Tissue pieces were then fixed in 1% PC-buffered glutaraldehyde at 4 °C overnight. The fixative solution was then replaced with PC-buffered 0.1 M-ammonium chloride and after 15 min at 4 °C tissues were dehydrated with a graded methanol series and embedded in Lowicryl HM20 as described by Lin & Langenberg (1983).

Affinity-purified goat anti-mouse IgG (Sigma) was used to prepare gold-labelled goat anti-mouse IgG complexes (Lin & Langenberg, 1983). Ultrathin sections of Lowicryl HM20-embedded tissue were treated with mouse anti-PYDV antiserum diluted 1/200 as a primary label followed by incubation with gold-labelled goat anti-mouse IgG as a secondary label according to Lin & Langenberg (1983). After immunogold staining, sections were stained in uranyl acetate and lead citrate (Reynolds, 1963) and viewed in a Zeiss 109 electron microscope at 80 kV.

Purified PYDV preparations used to immunize mice were analysed by SDS-polyacrylamide gel electrophoresis. All preparations contained five major structural proteins with mol. wt. of 190000 (190K), 84K, 52K, 31K, and 26K corresponding respectively to proteins L, G, N, M1 and M2 (Knudson & Macleod, 1972; Falk & Weathers, 1983; Adam & Hsu, 1984) (Fig. 1a). Comparison of colloidal gold-stained blots (Fig. 1b) and immunolabelled blots (Fig. 1c) showed that all five major proteins were labelled, indicating that the anti-PYDV antiserum reacted with all five structural proteins of PYDV. Protein L was seen on the original blots, but does not appear in Fig. 1(b) and (c).

In thin sections of PYDV-infected tobacco cells, aggregates of virus particles were commonly observed at the periphery of nuclei. As described by Black (1970) and Francki et al. (1981, 1985), virus particles aligned with their axes perpendicular to the nuclear membrane were often observed at an early stage of virus multiplication. At a later stage of infection, the particles increased in number to form virus aggregates that surrounded the nucleus (Fig. 2a). Perinuclear spaces were often distorted by accumulations of virus particles. Chromatin was less abundant in these nuclei than in healthy nuclei. There was a decreased intensity of staining and the fine, uniform appearance of nucleoplasm was absent. Instead, a granular matrix almost filled each nucleus (Fig. 2a). However, nucleoli did not degenerate in infected cells even when large aggregates of virus particles were observed.

After immunogold staining, gold particles were located over virus particles at the periphery of nuclei and predominantly over the granular matrix in the nucleoplasm (Fig. 2b), even at early
Fig. 1. (a) Coomassie Brilliant Blue-stained SDS-PAGE pattern of purified potato yellow dwarf virion polypeptides. Lane 1, marker proteins: myosin, β-galactosidase, phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and α-lactalbumin. Lane 2, proteins from purified intact virions. (b, c) Nitrocellulose blots of proteins from purified PYDV stained with a colloidal gold (b) or immunolabelled (c).

stages of infection (data not shown). Neither heterochromatin nor nucleoli were ever labelled. Occasionally, virus particles were present in nuclei, presumably because of invagination of the perinuclear space, and were also labelled. In apparently non-infected cells adjacent to infected cells, as well as in healthy uninfected controls, nuclei appeared to contain normal chromatin structures such as interdispersed heterochromatin and euchromatin. No virus particles were observed and no gold label was detected (Fig. 2c). Control sections were also not labelled when diluted preimmune serum was used (data not shown). Similar results were obtained with the two species of Nicotiana used in this study.

Our results clearly show that the nucleoplasm of PYDV-infected cells was composed largely of viral structural proteins and becomes the site of protein accumulation. This result supports the notion that the nucleocapsids, after acquiring their proteins in the nucleoplasm, acquire their envelopes from the inner nuclear membrane while passing from the nucleoplasm into the perinuclear space where assembled virus particles appear (Francki et al., 1985). In some instances small virus aggregates were also observed in the cytoplasm. However, no accumulation site other than the nucleoplasm has been found for viral structural proteins. Enveloped particles after acquiring their proteins in the nucleoplasm may enter the lumen of the endoplasmic reticulum (Francki, 1973) which is considered to be a continuation of the perinuclear space (Clowes & Juniper, 1968). Therefore, the nucleoplasm seems to be the only accumulation site for PYDV structural proteins in infected cells. At the present time we are unable to examine the sequence of acquisition of PYDV structural proteins in the assembly of virus particles. Monoclonal or monospecific antibodies prepared to individual structural proteins will be necessary for selective identification of the protein pool in the nucleoplasm of infected nuclei.

We are grateful to W. G. Langenberg for a critical reading of the manuscript, Mr Yuh-Kun Chen and Mrs Nancy Wang for their technical assistance. Published as paper No. 322, Scientific Journal Series of the Institute of Botany, Academia Sinica, Taipei, Taiwan, Republic of China.
Fig. 2. Electron micrographs of immunogold-stained sections of N. rustica leaf cells. Abbreviations used: Ch, chloroplast; Cr, chromatin; Cy, cytoplasm; Np, nucleoplasm; Nu, nucleus; Nuo, nucleolus; V, virus particles; Va, vacuole. All bar markers represent 200 nm. (a, b) PYDV-infected cells, (b) is an enlargement of part (a). (c) Non-infected cell near the infected one in (a).

REFERENCES


Short communication


(Received 2 March 1987)