Detection of the trans activity of the plum pox virus NIA-like protease in infected plants

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The NIA-like protein of plum pox virus is a protease with high sequence specificity that is autocatalytically released from the viral polyprotein. In order to determine whether the protease is active in trans we constructed a fusion protein consisting of the C-terminal region of the plum pox virus polyprotein and the staphylococcal Protein A. The authentic protease recognition sequence Asn-Val-Val-Val-His-Gln-Ala occurs in the centre of this protein fusion. This protein was cleaved specifically by extracts of plum pox virus-infected plants due to the strong activity of the viral protease making it a useful tool for diagnostic purposes.

The 9.8 kb messenger-sense RNA genome of plum pox virus (PPV), a plant potyvirus, is encapsidated in long flexuous rod-shaped particles by coat protein units of 36K (Mattanovich et al., 1988). The genome organization is very similar to that of the potyviruses tobacco etch virus (TEV) and tobacco vein mottling virus (TVMV) (Dougherty & Carrington, 1988). It contains a single open reading frame with the potential to encode a 354K polyprotein (Maiss et al., 1989). The mature viral proteins arise by processing of this large polyprotein precursor.

The NIA-like protein gene of PPV has been recognized as a potential protease by amino acid comparisons with known proteases (Maiss et al., 1989; Wellink & van Kammen, 1988) and by amino acid sequence comparison with the genes mapped on TEV (Carrington & Dougherty, 1987) and TVMV (Hellmann et al., 1988). The specific autocatalytic release of the PPV protease from a recombinant PPV polyprotein precursor cloned in Escherichia coli has been shown by Garcia et al. (1989a, b).

In order to demonstrate that the protease is active not only in cis but also in trans, we designed a protein (SPACP1) that contains the authentic, natural cleavage site for the NIA-like protein. This fusion protein consists of a part of the staphylococcal Protein A (SPA) of pRIT2T (Nilsson et al., 1985) connected to the PPV coat protein region and including the junction with the NIB-like protein. The junction contains a recognition sequence (Asn-Val-Val-Val-His-Gln-Ala) for the NIA-like protein (Fig. 1). The construction details are shown in Fig. 2.

The use of the plasmid pRIT2T in E. coli N4830 allowed heat induction of the fusion protein expression (Nilsson et al., 1985). The fusion protein was purified after lysis of the bacteria by affinity chromatography on human IgG-Sepharose 6FF (Pharmacia) according to the protocol provided by the manufacturer. The purified product was lyophilized and subsequently dissolved (at a concentration of 1 mg/ml) in phosphate-buffered saline (PBS), pH 7.4 containing 1 mM-dithiothreitol (substrate solution).

For the cleavage reaction, leaves of infected and non-infected Nicotiana clevelandii were independently ground in the substrate solution (1:4, w/v) and incubated for 5 min at room temperature. These samples were clarified by centrifugation and electrophoresed on an SDS-polyacrylamide gel (gradient 8 to 25%; Phast System, Pharmacia). The gel was blotted onto nitrocellulose, the membrane was blocked with 5% bovine serum albumin (BSA) in PBS and reacted with human IgG and then anti-human IgG–alkaline phosphatase conjugate in PBS containing 0.1% Triton X-100 (TPBS). After washing with TPBS, the membrane was stained with 0.3 mg/ml nitroblue tetrazolium and 0.2 mg/ml 5-bromo-4-chloro-3-indoxyl phosphate in 150 mM-veronal-acetate buffer pH 9.6 with 4 mM-MgCl₂.

Fig. 3 shows the results of a typical reaction after 5 min. The SPA part of the protein is released from the fusion protein after incubation with the extract of infected plants and can be traced in the Western blot as a 31K band (lane 1), whereas the sap of non-infected plant leaves does not substantially cleave the 67K fusion protein.
Short communication

Fig. 1. Schematic representation of the fusion protein SPACP1. The PPV NIa protease recognition sequence is located in the centre of the construct to give a detectable Protein A of 31K after cleavage.

Fig. 2. Construction of the pRITCP1 fusion protein vector. pPPV-NAT65 (Mattanovich et al., 1988) contains the cDNA of the 3' region of the PPV RNA. The coat protein (CP) region including the NIa recognition site was prepared by excision of the 1154 bp fragment with HaeIII and HindIII. The fragment was treated with calf intestinal phosphatase (CIP), blunt-ended with the Klenow fragment of DNA polymerase and ligated into the multiple cloning site (MCS, BamHI-linearized) of the blunt-ended and dephosphorylated pRIT2T. The vector was transformed into E. coli N4830-1.

Fig. 3. Western blots of SDS-polyacrylamide-electrophoresed plant material (N. clevelandii, 3 weeks post-infection). Lane 1, extract of PPV-infected leaves incubated with SPACP1; lane 2, extract of PPV-infected leaves; lane 3, extract of leaves from non-infected plants, incubated with SPACP1; lane 4, extract of PPV-infected leaves incubated with Protein A.

Fig. 4. Western blots of SDS-polyacrylamide-electrophoresed PPV-infected plant material in a time course reaction. Samples were taken for electrophoresis after the indicated time (min) of incubation with SPACP1.

Fig. 5. Western blot of leaf extracts of N. tabacum infected with tobacco etch virus (TEV) and potato virus Y (PVY) respectively, after 20 min reaction with SPACP1.

protein (lane 3). To show that the SPA itself is not cleaved by proteases in infected plants, we used SPA purified from pRIT2T-transformed E. coli and having an M\textsubscript{r} of 35K (lane 4). From the results in Fig. 3 we conclude that the PPV NIa-like protein has a strong proteolytic activity. The time course of the proteolysis is shown in Fig. 4. Complete cleavage occurs in this experiment within 6 minutes. The Western blot in Fig. 5 shows no degradation of the SPACP1 by tobacco etch virus or potato virus Y activity. We therefore conclude that the cleavage is PPV-specific.

Although the test was not quantitative, we think that this strong and specific cleavage of a PPV-specific fusion protein may find some application in the diagnosis of PPV-infected plants. With adaptation of the cleavage recognition sequence it may also be useful for the
detection of other virus infections. The described test procedure can be performed within 4 h and is therefore competitive with other test systems such as ELISA and nucleic acid hybridization tests.

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Note added in proof. We expressed the described 38K coat protein region in transgenic N. benthamiana and found evidence for trans activity of the NIa protease as only a 36K coat protein could be detected after infection with PPV.

References


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