A Comparison between the Capsid Proteins and the Products of in vitro Translation of Three Strains of Potato Virus X

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SUMMARY

The capsid proteins of three potato virus X isolates that belong to different strain groups on the basis of their reactions with host resistance genes have been compared by means of SDS-PAGE and partial proteolysis mapping. Considerable differences were observed in both the migration rates in SDS-PAGE and the peptide patterns generated after partial digestion with V8 protease of the capsid proteins of the isolates. The high molecular weight proteins synthesized by in vitro translation of genomic RNAs extracted from the three isolates were electrophoretically indistinguishable and antigenically unrelated to capsid proteins.

Previous studies have demonstrated that different strains of potato virus X (PVX) cannot readily be distinguished using antisera raised against PVX either by direct ELISA or latex agglutination tests (Wright, 1963; Fribourg, 1975; Moreira et al., 1980). Strains of PVX are usually classified on the basis of their interactions with host resistance genes (Cockerham, 1955, 1970) and although Torrance et al. (1986) have recently produced and characterized a range of monoclonal antibodies which are able to distinguish PVX strains, the serological classification does not correlate well with that of Cockerham (1955, 1970).

The PVX strains used in this study were isolates DX, HB and B (Jones, 1982; Moreira et al., 1980; Cockerham, 1955) which belong to different groups according to Cockerham’s classification. Isolate DX belongs to strain group 3 and is able to overcome potato hypersensitivity gene Nb but not hypersensitivity gene Nx (Jones, 1982), isolate B belongs to strain group 2 and is able to overcome gene Nx but not gene Nb (Cockerham, 1955), and isolate HB is a resistance-breaking strain that is able to overcome the immunity gene Rx in addition to genes Nx and Nb (Moreira et al., 1980). Virus particles were purified from each of the isolates as described previously (Adams et al., 1985) and the capsid proteins were examined by SDS-PAGE (Laemmli, 1970). The Mr of the major capsid proteins of the three isolates were different (Fig. 1a); isolate DX capsid protein had an Mr of 27500 ± 600 (1 s.e.) as compared to 28600 ± 300 (1 s.e.) for isolate HB and 29900 ± 400 (1 s.e.) for isolate B in 10 determinations. In addition, some preparations of each isolate also contained faster migrating minor bands with Mr of 25500 ± 300 (1 s.e.), 26000 ± 500 and 28700 ± 200 for isolates DX, HB and B, respectively (data not shown). The estimates for isolate DX correspond reasonably well with those reported (Koenig, 1972; Koenig et al., 1978) for the intact capsid protein (PVX-Ps) and its major cleavage product (PVX-Pf) generated by the action of a reducing agent-dependent protease of plant origin. However, although reports of differing Mr for various PVX preparations may sometimes be accounted for by the anomalous behavior of PVX coat protein in SDS-PAGE (Koenig, 1972; Koenig et al., 1978), the capsid protein subunits of isolated HB and B reproducibly migrated...
Fig. 1. (a) SDS-PAGE of the capsid proteins of PVX isolates HB (lane 1), DX (lane 2) and B (lane 3). Numbers indicate position and \( M_r \) \( \times 10^{-3} \) of protein markers. (b) Urea–SDS–PAGE of capsid proteins of isolates DX (lane 1), B (lane 3) and HB (lane 5) following partial proteolysis with V8 protease. Lanes 2 and 4 contain myoglobin and its CNBr fragments as standards (BDH) of \( M_r \) 16949, 14404, 8159, 6214 and 2512. An \( M_r \) 14000 to 15000 band produced by digestion of each isolate is arrowed. (c) Immunoblot of the V8 protease digestion products of capsid protein of isolates DX (lane 2), B (lane 4) and HB (lane 6) using MAC 72. Lanes 1, 3 and 5 contain 100 ng of undigested capsid protein of isolates DX, B and HB respectively. Numbers indicate the positions and \( M_r \) \( \times 10^{-3} \) of protein markers.

more slowly than those of isolate DX both in 10% polyacrylamide gels and 10 to 20% polyacrylamide gradient gels. Nevertheless although different mobilities between the three isolates might represent real differences in \( M_r \) between the capsid proteins, anomalous migration should not be discounted and an extended range of gel strengths should be investigated.

In order to investigate further the apparent differences observed between the three isolates the capsid proteins were subjected to partial proteolysis by \textit{Staphylococcus aureus} V8 protease as described by Cleveland \textit{et al.} (1977). Digestions were routinely performed using 0.5 mg/ml virus particles and 0-25 \( \mu \)g/ml V8 protease at 37 °C for 30 min in SDS-containing loading buffer followed by separation of the products by 12:5% SDS–PAGE in gels containing 8 M-urea (Swank & Munkres, 1971) (Fig. 1 b). The \( M_r \) and ranges of the major products of digestion were 14090 ± 750 (1 s.e.), 12010 ± 440 and 7570 ± 650 for isolate DX, 14970 ± 520 (1 s.e.), 12810 ± 510 and 10280 ± 940 for isolate B, and 15630 ± 400 (1 s.e.) and 9850 ± 650 for isolate HB. Although the mobilities of the largest band produced for each isolate (\( M_r \) 14000 to 15000) are similar, the sizes of the smaller fragments clearly differ. The observation of different mobilities of the proteolytic products reflects differences in the amino acid sequence among the three PVX capsid proteins. However, both the distribution and abundance of these amino acid alterations within the proteins remains to be determined.

In order to determine which, if any, of the fragments generated by V8 protease digestion of the three PVX capsid proteins shared a common epitope, the peptides were separated by 15% SDS–PAGE, electrophoretically transferred to nitrocellulose (Towbin \textit{et al.}, 1979) and probed with a monoclonal antibody, MAC 72, originally raised against isolate HB and known to react with all three isolates in indirect ELISA (Torrance \textit{et al.}, 1986). The second antibody was an anti-rat antibody conjugated to horseradish peroxidase (Miles Laboratories) and the enzymic reaction was developed as described by de Blas & Cherwinski (1983). Fig. 1(c) demonstrates that MAC 72 reacts strongly with a number of fragments generated by partial proteolysis of the proteins of all three isolates and that the sizes of the immunoreactive peptides are distinct. Because of differences in gel strength and composition Fig. 1(b) and (c) are not directly comparable and
only illustrate differences between smaller fragments in the former and larger fragments in the latter.

Using three other monoclonal antibodies (MAC 67, 68, 71), isolates HB and B produced characteristic patterns on immunoblots similar to those illustrated in Fig. 1(c). The differences were largely quantitative but whether these results reflect loss or destruction of (an) epitope(s), or cleavage at an epitope boundary thus reducing binding efficiency remains to be determined. Nevertheless the generation of different peptide patterns and the conservation of some common epitopes suggests differences in amino acid sequence between the three isolates.

We also investigated whether any differences between the isolates could be detected in the polypeptides translated in vitro from their genomic RNAs. RNA was extracted from purified virus preparations as described previously (Adams et al., 1985) and was translated at 0.2 μg/μl in rabbit reticulocyte lysate (Amersham) containing [3H]leucine according to the manufacturers’ instructions. Radioactively labelled translation products were separated by PAGE and detected by fluorography as described by Chamberlain (1979). Although numerous polypeptides were synthesized in each case, no protein that comigrated with the respective capsid proteins was ever detected. The in vitro translation products of DX RNA are shown in Fig. 2(a). Since the major products of in vitro translation detected for each isolate had M₉ in excess of 70000, the products generated after 60 min in vitro translation were compared following extended electrophoresis through a 7.5% SDS-polyacrylamide gel to improve resolution. Fig. 2(b) demonstrates that there are no detectable differences in size between the high M₉ proteins translated in vitro from RNA from each of the three isolates; the two largest polypeptides synthesized were estimated to have M₉ of 170000 and 140000. The observation that genomic PVX RNA fails to direct the synthesis of PVX capsid protein is in agreement with those of Ricciardi et al. (1980) and Wodnar-Filipowicz et al. (1980). Furthermore, the high M₉ polypeptides generated by translation of genomic DX RNA did not react with either a
polyclonal antiserum raised against isolate DX or the monoclonal antibody, MAC 72 (data not shown).

Although in vitro translation of genomic PVX RNA does not generate any detectable PVX capsid protein, the synthesis of a protein that is electrophoretically and immunologically identical to PVX capsid protein has been detected following in vitro translation of polysomal RNA isolated from DX-infected Nicotiana glutinosa tobacco plants (data not shown). However, the strategy by which PVX mRNA molecules active in capsid protein synthesis are generated is not known. Further investigation into the RNA content of polysomes from both infected plants and protoplasts may give indications as to the nature of the synthesis of PVX capsid in vivo.

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