Selective Proteolytic Activity Associated with Purified Sowthistle Yellow Vein Virus Preparations

(Accepted 17 February 1976)

SUMMARY

A proteolytic activity has been found associated with purified preparations of sowthistle yellow vein virus. The activity selectively digests the M1 and M2 proteins after dissociation of the virus with Nonidet P 40 (NP40), and is not stopped by several inhibitors of proteases.

Proteolytic activity has previously been demonstrated in association with purified erythrocyte membranes (Tokes & Chambers, 1975), several insect viruses (Eppstein & Thoma, 1975; Kozlov, Sidorova & Serebryani, 1975; D. Peters & R. M. D. Aalbers, personal communication), and some purified animal and plant viruses (Koenig et al. 1970; Hull, 1971; Pereira & Skehel, 1971; Holland et al. 1972; Huttinga & Mosch, 1974). We report the demonstration of a specific proteolytic activity associated with purified preparations of sowthistle yellow vein virus (SYVV), a rhabdovirus replicating in both plant and insect tissue.

SYVV was purified by a modification of the method of Peters & Kitajima (1970); magnesium acetate was used instead of magnesium chloride in solutions A and B and the low speed centrifugation was extended to 10 min. Macaloid (Internatio. Alg. en Chem. Handelsmij., Rotterdam), about 20 mg/ml in 0.01 M-tris/HCl buffer, pH 7.4, was added to the 8000 g supernatant fluid to a final concentration of about 1 mg/ml before Celite filtration. The final purified virus pellet obtained after zonal electrophoresis was resuspended in a small volume of freshly double distilled water. Virus concentration, in terms of protein, was determined by the method of Lowry et al. (1951).

Fig. 1 shows the protein band patterns obtained following overnight incubation of purified virus in buffer (a) and buffer plus NP40 (b). Incubation in the presence of the non-ionic detergent consistently resulted in selective degradation of two structural proteins, designated M1 and M2 in view of their possible membrane association. The proteolytic digestion was a slow process, no loss of M1 and M2 being detected after 1 h. Incubation in the presence of sodium azide (0.1 % to 0.001 %) had no effect on the proteolytic activity. The activity was not inhibited by a selection of protease-inhibitors added to the incubation mixture: phenyl methylsulphonyl fluoride, p-chloromercuribenzoic acid, ⍺-aminohexoic acid (all used at a final concentration of 10 mM), soybean trypsin inhibitor, kallikrein trypsin inhibitor (both used at final concentrations of 1 mg/ml and 0.1 mg/ml).

Proteolytic activity has previously been demonstrated in association with purified preparations of several membrane bounded viruses (Holland et al. 1972). However, the activity described here differs with respect to its specificity for two of the membrane associated proteins M1 and M2, which are degraded whereas the G and N proteins appear to be unaffected. This observed resistance of G and N to digestion may be conferred by the polymeric states of these proteins following NP40 dissociation of SYVV; the N protein remaining complexed with the nucleic acid (unpublished observation) and the G protein probably existing in the form of intact projections (manuscript in preparation). The proteolytic activity demonstrated
Fig. 1. Purified virus (30 to 50 μg) was incubated at room temperature (22 to 25 °C) for 15 to 20 h with an equal vol. of either (a) 0·1 M-PO₄ buffer, pH 7·6, or (b) 0·1 M-PO₄ buffer, pH 7·6, containing 0·4 % NP₄₀ (Shell Chemicals Ltd). Virus was disrupted by bringing the reaction mixture to 1 % with SDS and boiling for 5 min. Disrupted samples were electrophoresed on 7·5 % acrylamide gels, stained and destained (Weber & Osborn, 1969).

... in purified influenza virus and vesicular stomatitis virus preparations degrades the N proteins less effectively than the other proteins of these viruses (Holland et al. 1972).

The proteolytic digestion apparent in Fig. 1 was coupled with the appearance of one or possibly two high mol. wt. components (arrowed 1 and 2). However, these appear to be generated by the NP₄₀ dissolution of the membrane, being observed in preparations incubated for too short a time to be the result of proteolytic activity. The lower mol. wt. protein of the two (arrow 2) contains carbohydrate as determined by periodic Schiff’s reagent staining (J. L. Dale, personal communication), has an approximate mol. wt. of 150 000, and may represent a dimer of G protein. The nature of the upper band (arrow 1) is unknown. Aggregation of glycoproteins following SDS-disruption has been observed previously (Tuech & Morrison, 1974).

Protein components of mol. wt. lower than protein M₂ (arrows 3 and 4) were often observed following proteolytic digestion, and probably represent breakdown products of the two susceptible proteins. This observation suggests that low mol. wt. minor proteins detected with some purified rhabdovirus preparations may result from proteolytic cleavage of structural proteins (Knudson, 1973).

We have no data as regards the origin or location of the proteolytic activity, indeed we do not know whether we are dealing with one or several proteolytic enzymes. Because it is impossible to ensure absolute purity of a virus preparation, it is quite feasible that the activity is a contaminant located either externally or internally in a free or bound form. Alternatively it may be intrinsic to the virus and play a role in the infection process, or in the generation of biological activity of one or more of the structural proteins.

Irrespective of the origin or location of the proteolytic activity, these observations are pertinent with respect to the evaluation of experimental procedures involving biochemical
Short communication 453

studies on dissociated virus components. Such activity may be responsible for the inability to demonstrate virus associated transcriptase activity with several rhabdoviruses (Knudson, 1973; M. G. Schultz, R. MacLeod, personal communications), a procedure involving NP40 disruption of virus. This observation perhaps has particular relevance following the recent demonstration that minor structural proteins are required for the replicate activity of vesicular stomatitis virus (Emerson & Yu, 1975; Imblum & Wagner, 1975). Although the SYVV proteolytic activity acts slowly with respect to total digestion of structural components, the transcriptase, if present, may be more susceptible and its activity destroyed more quickly. A minor modification, occurring quickly and having little effect on the size and amount of any protein, may be sufficient to destroy biological activity.

Proteolytic activity associated with purified virus preparations may account for variations in the molar ratios of structural proteins observed with some rhabdoviruses. In the case of lettuce necrotic yellows virus (Francki & Randles, 1974), such activity may account for the low amounts of structural proteins other than G and N. The presence of proteolytic activity in purified virus preparations may prove to be a widespread phenomenon, particularly in the case of enveloped viruses.

A. Ziemiecki would like to acknowledge the International Agricultural Centre, Wageningen and the Agricultural University, Wageningen for research fellowships.

Laboratory of Virology A. ZIEMIECKI
Agricultural University D. PETERS
Binnenhaven 11
Wageningen
The Netherlands

REFERENCES

Short communication


(Received 15 December 1975)