An Inhibitor-resistant Protease Specified by an Insect Picornavirus, and the Role of Cellular Proteases in the Rapid Processing of Capsid Protein Precursors

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SUMMARY

Processing of cricket paralysis virus capsid protein precursors in vitro was resistant to the effects of a number of protease inhibitors. Leupeptin was effective in preventing cleavage of capsid protein precursors, suggesting that the virus-specified protease may be a serine protease. Virus capsid proteins were produced more rapidly in vitro when rabbit reticulocyte lysate was supplemented with an extract of Drosophila melanogaster cells. This suggests that cellular proteases may be involved in rapid processing of high molecular weight capsid protein precursors.

Cricket paralysis virus (CrPV) is one of a number of viruses isolated from insects and which have been classified as picornaviruses (Matthews, 1982). Classification of these viruses has been based upon biophysical properties such as buoyant density, morphology, size and nature of nucleic acid and proteins (Moore & Tinsley, 1982). Examination of the mechanism of replication of CrPV has demonstrated apparent discrepancies between CrPV and the replicative process proposed for picornaviruses. The RNA of picornaviruses functions as a polycistronic mRNA with protein synthesis being initiated at a single site. Stable protein products are formed by post-translational cleavages of a polyprotein which represents the entire coding capacity of the RNA (Sangar, 1979). The major proteins labelled with short pulses of $^{35}$S-methionine in CrPV-infected cells are two of the capsid proteins (VP1 and VP3) and the immediate precursor (VP0) of the other capsid protein (VP2), with relatively small amounts of larger precursors being detected (Moore et al., 1980). This is different from the situation found when cells are infected with mammalian picornaviruses, in which case large amounts of capsid proteins are produced only after processing of precursors. Synthesis of VP1 and VP3 is also resistant to the effects of a range of protease inhibitors which would be expected to prevent cleavage of capsid protein precursors (Moore et al., 1981a, b). This evidence suggests that VP1 and VP3 are synthesized by rapid cleavage of precursor proteins, and that the mechanism of protein synthesis by CrPV may differ fundamentally from that of picornaviruses. We have, however, demonstrated that CrPV virion RNA functions as a mRNA in vitro to give capsid protein precursors which are subsequently cleaved to give VP1 and VP3 (Reavy & Moore, 1981b). We report here an examination of the effects of protease inhibitors and Drosophila cell extracts on the synthesis and processing of CrPV-specified proteins in vitro, and conclude that the apparent differences exhibited by CrPV are due to the existence of an inhibitor-resistant virus-specified protease and rapid processing of high molecular weight precursors by cellular proteases.

RNA was extracted from purified CrPV by proteinase digestion and phenol extraction (Reavy & Moore, 1981a). To determine the effects of protease inhibitors on processing of CrPV-specified proteins, translation was performed for 30 min in rabbit reticulocyte lysate and subsequent processing of proteins was observed for 6 h in the presence of different inhibitors.

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Fig. 1. Processing of CrPV-specified proteins in the presence of various protease inhibitors. Translation was initiated in an assay comprising 40 µl lysate, 8 µl [35S]methionine (12.08 µCi/µl, 1010 Ci/mmol) and 2 µl RNA. Translation proceeded for 30 min and 5 µl of chase solution containing excess methionine and ribonuclease T₁ (Reavy & Moore, 1981b) was then added. A 5 µl sample was immediately removed and boiled for 2 min in 100 µl solubilizer (Moore et al., 1980) (a). The assay was then divided into 5 aliquots and incubated for 6 h in the presence of 1 µl water (b), 1 mM-phenylmethylsulphonyl fluoride (c), 1 mM-pepstatin (d), 1 mM-iodoacetamide (e) or 1 mM-leupeptin (f). Proteins were solubilized and electrophoresis was performed on a 12.5% polyacrylamide gel which was processed for fluorography. High molecular weight proteins 1 to 5 are labelled.

Fig. 2. Synthesis and processing of CrPV-specified proteins in untreated lysate (a to c) and in lysate supplemented with Drosophila cell lysate (d to f). Translation was initiated in assays consisting of 25 µl lysate, 5 µl [35S]methionine (12.08 µCi/µl, 1010 Ci/mmol), 1 µl of CrPV RNA and 2 µl sterile deionized water or Drosophila cell lysate. Samples (1 µl) were removed at 0, 15 and 30 min to monitor protein synthesis, and after 30 min incubation 3.5 µl of chase solution was added. Samples (5 µl) were removed at 0 h (a, d), 1 h (b, e) and 24 h (c, f) after chasing, and solubilized in 100 µl of solubilizer. Samples of 30 µl (solubilized products) were analysed by electrophoresis on a 12.5% polyacrylamide gel which was subsequently processed for fluorography. High molecular weight proteins are labelled 1 to 5.
Short communication

1833

(see Barrett, 1977 and Pelham, 1979 for action of inhibitors). Fig. 1 shows the results of such an experiment using pepstatin, iodoacetamide, phenylmethylsulphonyl fluoride (PMSF) and leupeptin. In the absence of inhibitors, an increase in the amounts of VP1 and VP3 was apparent after 6 h (Fig. 1 b) when compared with the proteins labelled after 30 min (Fig. 1 a). PMSF had no effect on the accumulation of VP1 and VP3 (Fig. 1 c) and little inhibition was observed with pepstatin (Fig. 1 d) or iodoacetamide (Fig. 1 e). Leupeptin, however, was particularly effective in preventing an accumulation of VP1 and VP3 (Fig. 1 f).

The rate of formation of the capsid proteins of CrPV in vitro is much slower than that observed in infected cells where they are the major products with short pulses of [35S]methionine. It is therefore possible that Drosophila cellular proteases play a part in processing of the viral precursor polypeptides. This possibility was examined with CrPV by performing translation in rabbit reticulocyte lysate supplemented with a lysate of Drosophila cells as a source of proteases. Fig. 2 shows the proteins synthesized and their subsequent processing in the presence and absence of a Drosophila lysate supplement. The proteins synthesized appeared to be unchanged in the presence of the Drosophila lysate and the same high molecular weight proteins were synthesized (Fig. 2 a, d). The processing of proteins in supplemented lysate differed from that observed in untreated lysate in a number of important respects. Processing of proteins 2 and 4 was much more rapid in the supplemented lysate, with protein 2 decreasing in amount within 1 h of chasing (Fig. 2 b, e). Also, a marked decrease in the amount of protein 4 was observed within 24 h of chasing, whereas it was apparently stable in untreated lysate (Fig. 2 c, f). Protein 5 was also processed much more rapidly in the supplemented lysate, and a concomitant increase in a protein which co-migrated with VP0 was observed (Fig. 2 f). Protein 5 was much more stable in the untreated lysate, suggesting that its cleavage is caused by a Drosophila protease (Fig. 2 c).

Synthesis of the CrPV capsid proteins is carried out in vitro by a virus-specified protease (Reavy & Moore, 1983). The appearance of VP1 and VP3 in the presence of protease inhibitors indicates that the CrPV-specified protease is resistant to the effects of such inhibitors. Leupeptin did have an effect on the synthesis of VP1 and VP3, suggesting that the CrPV protease is possibly a serine protease. Although the CrPV-specified protease is able to cleave capsid protein precursors in vitro, these cleavages appear to be performed more efficiently by Drosophila proteases.

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REFERENCES


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