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Adenovirus protease expressed in insect cells cleaves adenovirus proteins, ovalbumin and baculovirus protease in the absence of activating peptide

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Abstract

The adenovirus type 2 protease (EP) was expressed by infecting insect cells with a recombinant baculovirus. Immunoblot and activity analysis showed EP to be present in both the nucleus and cytoplasm. While the insect cell expressed EP was more soluble than the *Escherichia coli* expressed EP, its activity was one quarter of the latter, suggesting that eukaryotic postsynthetic modifications are not essential for enzyme activity. EP inactivated a cytoplasmic cathepsin-like baculovirus-encoded cysteine protease which carries a single EP cleavage site and which was capable of digesting most adenovirus structural proteins in vitro. In addition to cleavage of the baculovirus protease, the adenovirus EP was also able to cleave ovalbumin and canine adenovirus protein pre-VII, in the absence of activating peptide. EP activation therefore may occur by means of factors other than the specific activating peptide.

Keywords: Adenovirus protease; Baculovirus protease; Protease; Cysteine

1. Introduction

Adenoviruses encode a cysteine protease (EP) that is essential for infectious virus production (Weber 1976, 1990, 1994). The enzyme cleaves viral proteins IIIa, pTP, pre-VI, pre-VII, pre-VIII, L2-11K (pre-Mu), L1-52K and host cytokeratins

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K7 and K18 at consensus sites (M,I,L)XGG-X or (M,I,L)XGX-G (reviewed in Weber 1990, 1994; Weber and Tihanyi, 1994). Although a great deal is already known about the protease and its function in virus replication, a number of questions concerning the properties of the enzyme remain unresolved or controversial. One of these questions is the apparent requirement by the enzyme for a specific activating peptide (Mangel et al., 1993; Webster et al., 1993). In our experiments, no such requirement could be demonstrated (Tihanyi et al., 1993). The enzyme appeared to be fully active in the absence of this peptide. It is conceivable that differences in experimental procedures, such as the source and methods of preparation of the enzyme or the demonstration of its enzymatic activity are the cause of these disagreements. Our studies had been based on EP expressed in *Escherichia coli*. Here we report the expression of EP and its consequences in insect cells by means of a baculovirus mediated expression system.

2. Materials and methods

2.1. Construction of recombinant baculovirus expressing the adenovirus protease

The protease gene was amplified from the Ad2 *Hinc*II-J fragment by means of the polymerase chain reaction (PCR) using the following primers: 5′-CTCGCTAGCCATGGCCTCCAGTGACG-3′ and 5′-CCGCTAGCTTACATGTTTTTCAAGT-3′. The 645-bp PCR product was purified, digested with *Nhe*I and ligated into the unique *Nhe*I site of the pETL transfer vector (a gift from C. Richardson, BRI, Montreal) so expression of the protease gene would be under the control of the baculovirus polyhedrin promoter (Vialard et al., 1990). The plasmid was amplified in bacteria and both sense (pETL-Ps) and antisense (pETL-Pas) orientations isolated. *Autographa californica* Nuclear Polyhedrosis Virus (AcMNPV) derivative AcRP6-SC (Kitts et al., 1990) DNA (750 ng) linearized with *Bsu*36I and pETL-Ps or pETL-Pas DNA (3 μg) were cotransfected into *Spodoptera frugiperda* (Sf9) cells (purchased from Invitrogen) using lipofectin (GIBCO-BRL). Blue plaques were picked and purified by two rounds of plaque purification as previously described (Carstens et al., 1993).

2.2. Purification of adenovirus protease

Purification of EP was carried out as described previously, but with the following modifications (Weber and Tihanyi, 1994). In place of lysozyme, cells were disrupted by 5 cycles of freeze–thawing followed by sonication (20 times 5 s each). This modification eliminated contamination by lysozyme which tends to copurify with EP. The DEAE-Sephacel-treated solution was centrifuged at 15,000 rpm for 15 min. The pH of buffer B was increased to 6.8. This protocol generally yielded more than 1 mg of enzyme per liter of cells at an estimated 98% purity. Purified EP was either used fresh or stored bound to carboxymethyl cellulose to increase its shelf-life and eluted as needed with Tris-HCl (0.2 M, pH 9) containing 0.2 mM EDTA, 5 mM mercaptoethanol and 10% glycerol.

2.3. Proteinase assay

Proteinase activity was determined on 4 types of substrate: (1) [^{35}S]methionine-labeled purified disrupted Ad2 ts1 39°C virions, as described before (Tremblay et al., 1983), looking at the conversion of pre-VII to VII; (2) [^{35}S]methionine-labeled ts1-infected Hep2 cell lysate prepared at 39°C and boiled for 2 min to inactivate proteolytic enzymes; (3) purified, recombinant canine adenovirus type 1 pre-VII (Cai and Weber, 1993); (4) ovalbumin, grade V (Sigma) was denatured by boiling for 3 min, the native protein being resistant to digestion. The reaction mixtures (40 μl) generally contained 20 μl substrate, 5 μl (5 ng) enzyme or cellular fraction and 0.1 M Tris-HCl, pH 7.5, and was incubated overnight (18 h) at 37°C. Cleavage was detected by SDS-PAGE followed by staining with Coomassie R250 or silver, or by autoradiography.

2.4. Immunoblotting

Proteins separated by SDS-PAGE were electroblotted onto nitrocellulose Hybond C-extra membranes (Amersham) reacted with anti-EP or antibaculovirus protease serum and antigen–antibody complexes were detected with protein A labeled with ^{125}I (Amersham).

2.5. Reagents

Iodoacetamide was freshly prepared in distilled water at 10 mM concentration, pH adjusted to 7.5 with Tris. Peptide PVIc (GVQSLKRRRCF) (Coast Scientific, San Diego, CA) was HPLC purified and a stock solution (400 μM) prepared in 10 mM Tris, pH 7.8. Aliquots were frozen and used at a final concentration of 10 μM. Anti-EP was a polyclonal rabbit serum against the purified recombinant adenovirus type 2 protease. Anti-baculovirus protease antibody was also a polyclonal rabbit serum made by immunization with SDS-PAGE-purified recombinant protease and a generous gift from Jeff Slack, Queen's University, Kingston, Ontario. SVLT (obtained from C. Prives) is a recombinant baculovirus expressing the SV40 large T-antigen under the control of the polyhedrin promoter (O'Reilly and Miller, 1988). PLAM is a modified expression plasmid derived from pRIT2T (Pharmacia) by deletion of the protein A-coding segment and used here as a negative control for PLPV which is PLAM containing the adenovirus type 2 protease gene (Tihanyi et al., 1993).

3. Results

3.1. Cloning and expression of adenovirus protease

The PCR amplified adenovirus protease gene was cloned in the pETL transfer vector under the control of the polyhedrin promoter and cotransfected with AcRP6-SC into Sf9 cells. Recombinant baculoviruses were isolated and two of them (B1 and B2) were tested for the presence of the protease gene by PCR amplification by means of the same primers as used for the cloning. Both

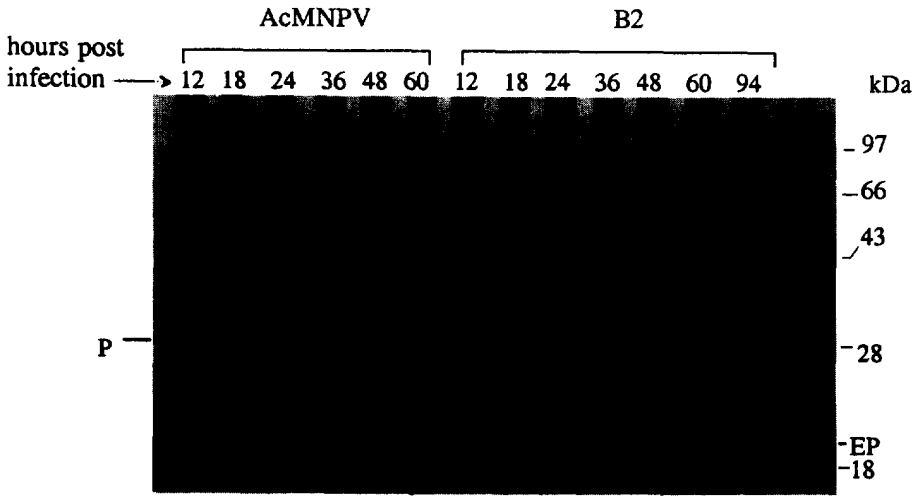

Fig. 1. Time course of protein expression. Sf9 cells were infected with wild-type baculovirus or B2 recombinant baculovirus containing the adenovirus proteinase gene. At different times postinfection, the cells were lysed and subjected to SDS-PAGE (12%) and stained with Coomassie blue. P, polyhedrin; EP, adenovirus endoproteinase.

recombinants contained a band of the expected 0.6 kbp size that comigrated with the amplification product obtained from adenovirus. Wild type and recombinant virus stocks were prepared in Sf9 insect cells and the time course and level of expression of the protease tested. Polyhedrin was visibly expressed in wild-type virus-infected cells by 24 h postinfection and it reached a maximal level by 48 h (Fig. 1). A new band which corresponds to the molecular weight of the protease (23 kDa) appeared in B2-infected cells at 48 h (Fig. 1). The later appearance of the presumed protease appeared to be due to a lower level of synthesis when compared with the viral polyhedrin. This could be due to toxicity of the enzyme in the Sf9 cells. B1-infected cells also expressed a new band which migrated as a 16-kDa protein (not shown).

To identify the adenovirus protease, cell fractionation and immunoblotting was carried out. Sf9 cells were infected with B2 virus, and as a negative control with SVLT virus which carries the SV40 large T-antigen. Two days after infection the cells were fractionated into nuclear and cytoplasmic fractions and the proteins separated by SDS-PAGE. The presumed EP was present in both fractions, but predominantly in the nucleus (Fig. 2, lanes c, d). Reaction of an immunoblot with a polyclonal antiserum to the EP confirmed the 23-kDa band as the adenovirus protease (Fig. 2, lanes h, i and l, m). Again, both nuclear and cytoplasmic fractions contained EP. The fractions are not visibly cross-contaminated as shown by the nuclear location of the 4 histones (Fig. 2, lanes a, c) and also by the nuclear location of a small baculovirus protein at the bottom of the gel (Fig. 2, lane c). The presence of the EP in the insect cell cytoplasm contrasts with the largely nuclear location of the EP in human cells infected by adenovirus (Bhatti and Weber, 1979).

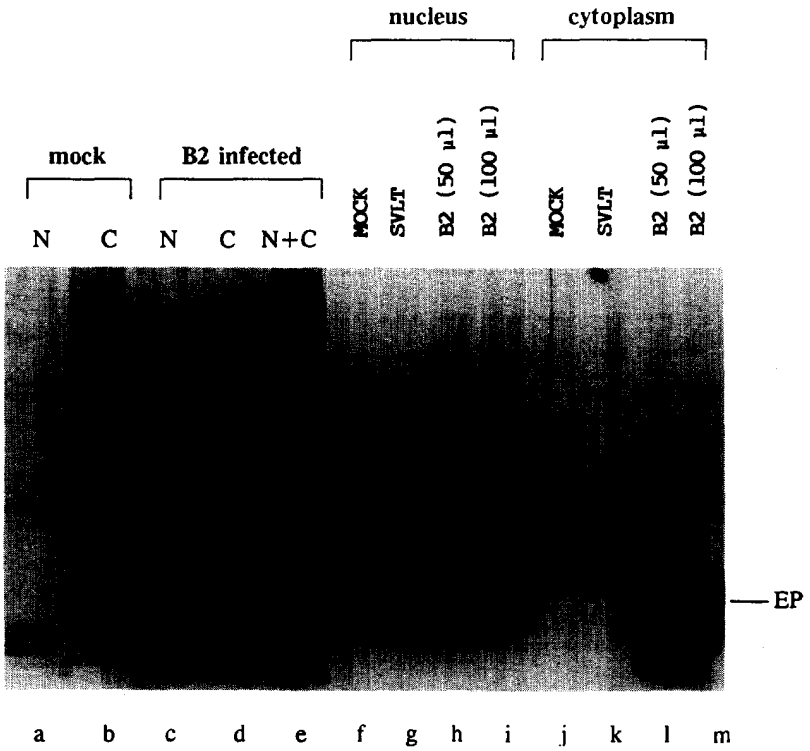

Fig. 2. Identification and cellular localization of the recombinant EP. Sf9 cells were infected with B2 (EP) or SVLT (control) recombinant baculoviruses and 72 h postinfection the nuclear and cytoplasmic fractions were separated using 0.1% NP40 as described (Bhatti and Weber, 1979) and subjected to SDS-PAGE. One gel was stained with Coomassie blue (a–e), another was blotted onto nitrocellulose and stained with anti-EP serum followed by [^{125}I]protein A (f–m).

The B1 virus produced a 16-kDa band by immunoblotting with antiprotease serum (not shown). It appears, therefore, to be a truncated protein presumably as a result of a PCR-induced mutation. For this reason all subsequent experiments were done with the B2 virus.

3.2. Protease activity

Protease activity of cell lysates was measured by the cleavage of viral substrate proteins, particularly pre-VII, in ts1 virions produced at the non-permissive temperature. Fig. 3 clearly shows the expected cleavage pattern usually obtained in this assay (lanes f, j). Furthermore, both the nuclear and cytoplasmic enzymes had cleavage activity. Also, as expected, the cysteine proteinase inhibitor iodoacetamide, completely abolished enzyme activity (lanes g, l). No enzyme activity was detected in mock-infected cells (lanes d, h) nor in the nuclear fraction of cells infected by the control baculovirus, SVLT (lane e). Surprisingly, SVLT-infected cytoplasmic extracts exhibited a protease activity which digested all but two adenovirus proteins (II and IV; lane i). The same activity was also observed in the cytoplasmic fraction of B1-infected cells and wild-type baculovirus-infected cells

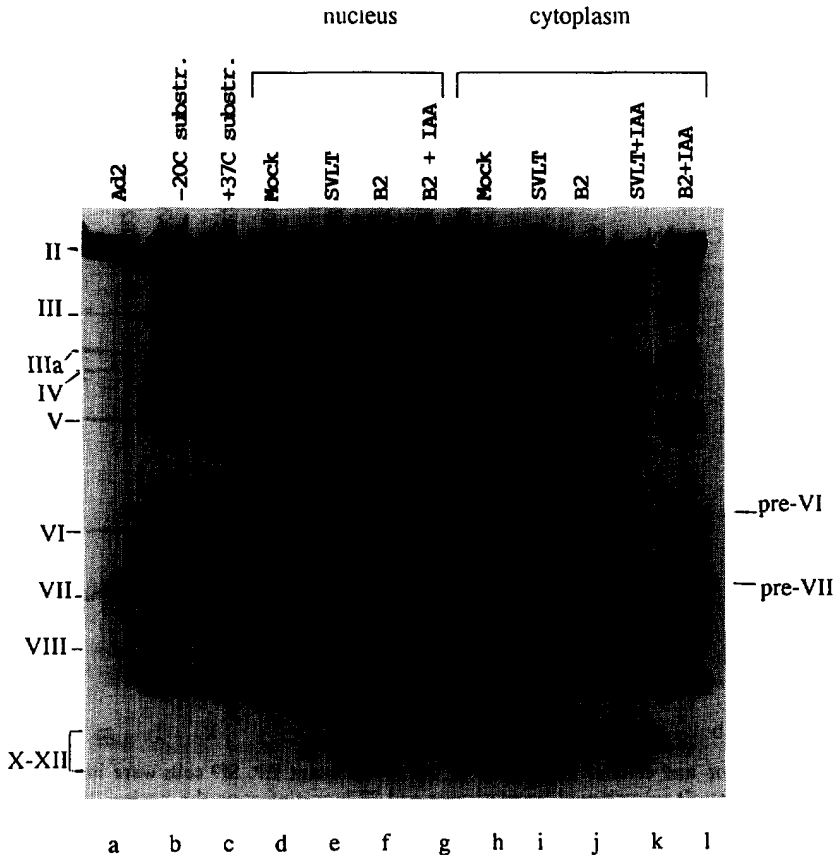

Fig. 3. Enzyme activity. Nuclear (c–g) and cytoplasmic (h–l) fractions from Sf9 cells 72 h postinfection were added to [^{35}S]Met-labeled adenoviral substrate proteins and incubated overnight at 37°C. The cysteine proteinase inhibitor, iodoacetamide (IAA) was added to a final concentration of 1 μM to some tubes (g, k, l). The reaction mixtures were separated by SDS-PAGE, dried and exposed. EP activity is evaluated by the cleavage of adenovirus protein pre-VII. Lane a, wild-type Ad2 marker; lanes b and c, substrated controls.

(data not shown). This protease activity was also sensitive to inhibition by iodoacetamide (lane k). It appears, therefore, that baculoviruses may express a thiol-like protease in the cytoplasm of Sf9 cells. Interestingly, this protease activity is only observable in SVLT-, B1- or wild-type virus-infected cells. B2-infected cells only show the adenovirus EP activity, as demonstrated by the characteristic cleavage of viral precursor proteins. Because the baculovirus protease activity is abolished only in the presence of the active adenovirus EP (B2 infection), but not in the presence of the inactive EP (B1 infection), one explanation might be that the adenovirus EP inactivates the baculovirus protease by proteolysis. A search of sequence databanks revealed a baculovirus protease sequence submission (M67451) which showed a high degree of homology to cathepsins (FASTA z-value of 10 or greater) The highest degree of homology was to *Trypanosoma brucei* cathepsin (sw: cysp – trybb) at 55% homology and 33% identity. This sequence contains a single adenovirus EP

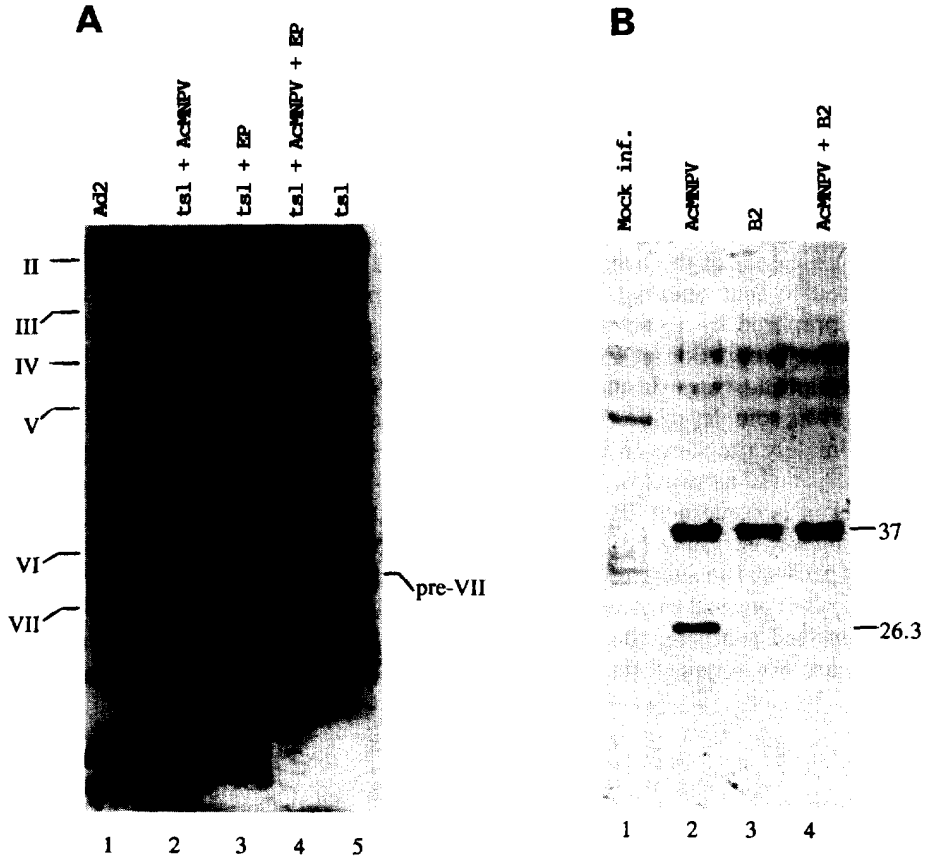

Fig. 4. Inactivation of baculovirus protease by the adenovirus EP. A: the cytoplasmic fraction of Sf9 cells infected for 48 h with AcMNPV was tested for enzyme activity on ts1 substrate proteins after prior incubation with EP. 1, Ad2 marker virus; 2, substrate incubated for 2 h with AcMNPV cytoplasmic fraction, note the extensive digestion of adenovirus proteins; 3, substrate incubated with purified recombinant EP (PLPV), note the cleavage of pre-VII to VII; 4, the AcMNPV cytoplasmic fraction was preincubated for 15 h with EP followed by a second incubation for 15 h with the ts1 substrate, note the complete abolition of the baculovirus protease activity; 5, ts1 substrate incubated without any additions. B: Sf21 cells were infected with AcMNPV and B2 recombinant baculovirus (m.o.i. of 10) or dual infected with AcMNPV and B2 (m.o.i. of 5 each). The cell lysates were tested for the presence of the baculovirus protease 48 h after infection by immunoblotting with polyclonal antisera directed against the baculovirus protease gene product. Lanes, 1, mock-infected Sf21 cells; 2, AcMNPV-infected Sf21 cells; 3, B2 recombinant-infected Sf21 cells; 4, AcMNPV- and B2 double-infected Sf21 cells. Note the digestion of the 26.3-kDa mature baculovirus protease in the presence of the adenovirus protease (B2 virus) in lanes 3 and 4.

cleavage site at residue 276, LVGY-G. It is therefore possible that the adenovirus EP inactivates the baculovirus protease by cleavage at residue 276. This hypothesis was tested directly by incubating a cytoplasmic extract of Sf9 cells infected with WT baculovirus with purified adenovirus EP (from *E. coli*). The results clearly demonstrated the inactivation of the baculovirus-specific protease activity by the adenovirus EP (Fig. 4A, lane 2 vs 4). Proteolytic cleavage of the baculovirus protease was also demonstrated by staining the enzyme with specific antibody in an

immunoblot of infected cell extracts (Fig. 4B). The antibody stained the full length translation product of the protease precursor (37 kDa or 324 amino acids) and the putative glycosylated C-terminal enzyme domain which is homologous to cathepsins (26 kDa or 210 amino acids) (Fig. 4B, lane 2). Interestingly, only the 26-kDa enzyme domain was digested by EP (Fig. 4B, lanes 3 and 4), the 37 kDa precursor being protected perhaps by the precursor moiety of the molecule.

3.3. Comparison of protease production in insect cells versus E. coli

The efficiency of the baculovirus vector-based expression system in Sf9 cells was compared to that obtained with the pRIT2T expression plasmid in *E. coli*. Cell lysates prepared by 5 cycles of freeze–thawing were centrifuged at 10,000 *g* and the relative proportion of EP in the pellet and supernatant estimated by means of immunoblotting. Approximately 75% of the Sf9 product, and 35% of the *E. coli* product was soluble, while the total enzyme concentration (per mg wet weight) was approximately the same in the two cell types. Enzyme activity of the supernatant was determined as usual by the cleavage of pre-VII to VII in ts1 substrate virions. The Sf9 cells contained 19 units/mg wet weight, while the *E. coli* contained 73 units/mg wet weight (a unit of enzyme was arbitrarily defined as the activity which cleaved 50% of the substrate during an 18-h incubation). As the enzyme activity of the *E. coli*-expressed protease was not inferior, but in fact 3.8-fold greater than the Sf9 expressed protease, these results suggest that eukaryotic postsynthetic modifications are not required for enzyme activity.

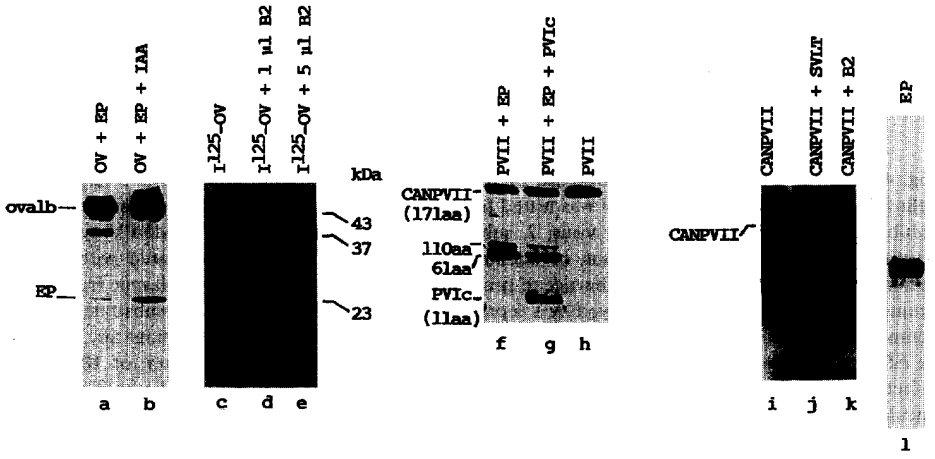

Fig. 5. Cleavage of ovalbumin and CanPVII by recombinant adenovirus proteases. a–b, cleavage of denatured ovalbumin (a) by purified EP produced in *E. coli* and its inhibition (b) by iodoacetamide (1 µM IAA), silver-stained SDS-PAGE; c–e, cleavage of [^{125}I]labeled ovalbumin by a nuclear-free B2-infected cell extract (autoradiogram); f–h, cleavage of purified recombinant canine adenovirus type 1 pre-VII (canPVII) protein by, (f) purified *E. coli* expressed EP, (g) purified *E. coli* expressed EP plus pVIc peptide, (h) substrate only control (silver-stained gel); i–k, cleavage of purified canPVII protein labeled with ^{125}I by, (i) substrate control only, (j) baculovirus protease (SVLT infected nuclear-free cell extract), and (k) adenovirus protease (B2-infected cell extract, autoradiogram). Lane l shows the protease (about 100 ng, silver stain) used in these experiments, estimated to be 98% pure. The reaction mixtures contained 1–5 ng protease.

3.4. Cofactors are not always required for adenovirus protease activity

Two recent reports have indicated that in the hands of the authors the adenovirus protease requires activation by an 11 amino acid cleavage product from viral protein pre-VI (Mangel et al., 1993; Webster et al., 1993). As the ts1 substrate virus used in our assays contains pre-VI, it might provide the peptide required for enzyme activation. Enzyme activity was therefore tested on two purified substrate proteins: (1) recombinant canine adenovirus pre-VII (canPVII); and (2) ovalbumin. Both proteins were cleaved by the B2 enzyme in the absence of cofactors (Fig. 5, lanes d, e, k). The fragments produced are consistent with cleavage at the LPGF-G site at residue 65 (P1) in ovalbumin and at the LFGG-A site at residue 61 (P1) in canPVII. The purified *E. coli* produced EP also cleaved purified canPVII in the absence of pVIc (Fig. 5, lane f). In some experiments, the addition of the pVIc peptide GVQSLKRRRCF, resulted in additional cleavage fragments. Enzyme activity in the absence of PVIc was observed in both fresh preparations, preparations which had been stored on CMC, or preparations which were purified without the CMC step, thus ruling out CMC as a potential activator. Therefore we conclude that the adenovirus EP produced in insect cells resembled the *E. coli*-produced EP in that it was active in the cleavage of substrate proteins and did not show a requirement for the pVIc activating peptide. It should be noted, however, that PVIc restored activity to aged enzyme preparations which had lost it. This suggests that our purification or experimental protocols favor the appropriate redox state of the protein, obviating the requirement for PVIc.

4. Discussion

Our results show that the adenovirus protease expressed in insect cells exhibits an enzyme activity comparable to that expressed in *E. coli* and that contrary to some reports the addition of activating cofactors is not required. Previous reports have demonstrated the synthesis of recombinant adenovirus protease in *E. coli* or insect cells, but a direct comparison of the two expression systems has not been reported until now. As we have obtained comparable enzyme activity in the two systems, the present results strongly support what has until now been an implicit assumption that eukaryotic-specific postsynthetic modifications at the RNA or protein level are not required for adenovirus protease activity. This conclusion is consistent with the paucity of conserved sequence motifs for postsynthetic protein modifications in the 12 protease sequences known to date (Weber and Tihanyi, 1994).

The second important conclusion which emerges from these studies is that the putative activating peptide GVQSLKRRRCF (pVIc) is not required for protease activity in vitro. This conclusion is consistent with our previous studies using purified protease made in *E. coli* (Tihanyi et al., 1993). The requirement for enzyme activation by pVIc was based largely on small peptide based enzyme assay systems (Mangel et al., 1993; Webster et al., 1993). Our assay was based on the cleavage of ovalbumin and pre-VII at known sites. Indeed, Webster, et al. (1993) also observed some cleavage activity with their enzyme when viral protein pTP was presented as the substrate. Recently we have demonstrated cleavage of a fluorescent peptide substrate by crude or purified recombinant protease in the absence of

activating peptide (unpublished observations). Three additional observations also call into question the proposed activation role of the pVIc peptide: (1) cytokeratins K7 and K18 have been reported to be cleaved by the EP (Chen, et al. 1993); (2) the L1-52K scaffolding protein appears to be cleaved by EP prior to the cleavage of any other viral protein, including pre-VI (Hasson, et al. 1992); and (3) we have shown here that the adenovirus EP expressed in the insect cell cytoplasm cleaves the cytoplasmic baculovirus protease, in the absence of any other adenovirus proteins (especially pre-VI). Combined with the other results described in this report, all these observation tend to argue against an absolute and exclusive requirement for protease activation by the pVIc peptide. It is possible that small differences in the purification or storage of enzyme or differences in the assay systems create the requirement for pVIc on the one hand and dispense with it on the other. It is also conceivable that certain cleavage events proceed without pVIc, while others require the presence of the peptide.The question clearly requires further study.

A surprising adventitious finding in the course of these experiments concerns a baculovirus encoded protease. Baculovirus-infected Sf9 cells expressed a potent cytoplasmic protease activity not found in uninfected Sf9 cells nor in cells infected by the B2 recombinant virus carrying the adenovirus protease. Most adenovirus proteins as well as ovalbumin were digested to small peptides and proteolysis appeared to be sensitive to iodoacetamide, an inhibitor of thiol proteases. Most or all of this proteolytic activity may be due to the baculovirus encoded thiol protease (Rawlings et al., 1992: Faulkner P., GeneBank Accession No M97906). The absence of detectable baculovirus-specific protease activity in B2-infected cells appears to be due to proteolytic inactivation of the baculovirus protease by the adenovirus protease. Cleavage probably takes place at the single adenovirus-specific protease consensus site LVGYG at residue 276 of the full length protein. This observation suggests two conclusions: (1) the adenovirus protease present in the cytoplasm is active in vivo on an insect virus protein, in addition to the demonstrated in vitro activity on adenovirus proteins and ovalbumin (without the need for pVIc mediated activation); and (2) the baculovirus protease is not essential for virus replication. The second conclusion is implicit in the fact that the B2 virus grows. It is likely that the baculovirus protease plays an important role in the insect host, perhaps in the process of tissue liquefaction which facilitates the release of polyhedra from the insect body. It is interesting to note in this regard that the structurally related cathepsins of mammals are also functional in tissue remodeling and degradation as the *Trypanosoma* proteases are functional in tissue invasion (Bond and Butler, 1987; McKerrow, 1989; Liotta and Stetler-Stevenson, 1991; Tardieux et al., 1992). That the protozoans' life cycle includes a stage within which insect hosts might have bearing on the evolutionary origin of these proteases. The broad cleavage specificity of this enzyme should be cause for concern in the expression applications of the virus and suggests the desirability of deleting this gene in baculovirus expression vectors.

Acknowledgements

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References

Bhatti, A.R. and Weber, J.M. (1979) Protease of adenovirus type 2: subcellular localization. J. Biol. Chem. 254, 12265–12268.

Bond, J.S. and Butler, E.P. (1987) Intracellular proteases. Annu. Rev. Biochem. 56, 333–364.

Cai, F. and Weber, J.M. (1993) Primary structure of the canine adenovirus PVII protein: Functional implications. Virology 193, 986–988.

Carstens, E.B., Lu, A.L. and Chan, H.B. (1993) Sequence, transcriptional mapping and overexpression of p47, a baculovirus gene regulating late gene expression. J. Virol. 67, 2513–2520.

Chen, P.H., Ornelles, D.A., Shenk, T. (1993) The adenovirus L3 23-kilodaltonproteinase cleaves the amino-terminal head domain from cytokeratin 18 and disrupts the cytokeratin network of HeLa cells. J. Virol. 67, 3507–3514.

Hasson, T.B., Ornelles, D.A., Shenk, T. (1992) Adenovirus L1 52- and 55-kilodalton proteins are present within assembling virions and colocalize with nuclear structures distinct from replication centers. J. Virol. 66, 6133–6142.

Kitts, P.A., Ayres, M.D. and Possee, R.D. (1990) Linearization of baculovirus DNA enhances the recovery of recombinant virus expression vectors. Nucleic Acids Res. 18, 5667–5672.

Liotta, L.A. and Stetler-Stevenson, W.G. (1991) Tumor invasion and metastasis: an imbalance of positive and negative regulation. Cancer Res. (Suppl.) 51, 5054s–5059s.

Mangel, W.F., McGrath, W.J., Toledo, D.L. and Anderson, C.W. (1993) Viral DNA and a viral peptide can act as cofactors of adenovirus virion proteinase activity. Nature 361, 274–275.

McKerrow, J.H. (1989) Parasite proteases. Exp. Parasitol. 68, 111–115.

O'Reilly, D.R. and Miller, L.K. (1988) Expression and complex formation of simian virus 40 large T antigen and mouse p53 in insect cells. J. Virol. 62, 3109–3119.

Rancourt, C., Tihanyi, K., Bourbonnière, M. and Weber, J.M. (1994) Identification of active-site residues of the adenovirus endopeptidase. Proc. Natl. Acad. Sci. U.S.A. 91, 844–847.

Rawlings, N.D., Pearl, L.H. and Buttle, D.J. (1992) The baculovirus autographa californica nuclear polyhedrosis virus genome includes a papain-like sequence. Biol. Chem. Hoppe-Seyler 373, 1211–1215.

Tardieux, I., Webster, P., Ravesloot, J., Boron, W., Lunn, J.A., Heuser, J.E. and Andrews, N.W. (1992) Lysosome recruitment and fusion are early events required for *Trypanosoma* invasion of mammalian cells. Cell 71, 1117–1130.

Tihanyi, K., Bourbonniere, M., Houde, A., Rancourt, C. and Weber, J.M. (1993) Isolation and properties of the adenovirus type 2 proteinase. J. Biol. Chem. 268, 1780–1785.

Tremblay, M.L., Déry, C.V., Talbot, B.G. and Weber, J. (1983) In vitro cleavage specificity of the adenovirus type 2 proteinase. Biochem. Biophys. Acta 743, 239–245.

Vialard, J., Lalumiere, M., Vernet, T., Briedis, D., Alkhatib, G., Henning, D., Levin, D. and Richardson, C. (1990) Synthesis of the membrane fusion and hemagglutinin proteins of measles virus, using a novel baculovirus vector containing the B-galactosidase gene. J. Virol. 64, 37–50.

Webster, A., Hay, R.T. and Kemp, G. (1993) The adenovirus protease is activated by a virus-coded disulphide-linked peptide. Cell 72, 97–104.

Weber, J.M. (1976) Genetic analysis of adenovirus type 2. III. Temperature-sensitivity of processing of viral proteins. J. Virol. 17, 462–471.

Weber, J.M. (1990) The adenovirus proteinase. Semin. Virol. 1, 379–384.

Weber, J.M. (1994) The adenovirus endopeptidase and its role in virus infection. Curr. Topics Micro. Immunol., 199/I, 227–235.

Weber, J.M and Tihanyi, K. (1994) Adenovirus endopeptidases. Methods in Enzym. 244(D), 595–604.